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Current Studies of the Pteridophyte Life Cycle

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I. Abstract

The factors controlling the gametophyte/sporophyte/gametophyte cycle in the Pteridophyta are re-examined in the light of current knowledge of gametogenesis, sporogenesis, apospory and apogamy.

The ultrastructural and cytochemical features of gametogenesis point to oogenesis as being particularly significant in relation to change of reproductive phase from gametophyte to sporophyte. The egg cell is richly endowed with cytoplasm, and it matures in a closed chamber (the archegonium), in which there is opportunity to take in nutrients released by the lysis of the other cells in the archegonial canal. The cytoplasm of the mature gamete is well provided with organelles and ribosomes, and contains substantial quantities of RNA and DNA. The latter is principally in the organelles, but a small amount may be free in the cytoplasm, accompanied by histones. The chromatin is finely dispersed, giving no detectable Feulgen reaction, and chromosomes cannot be recognized. It is suggested that the unique enrichment of the cytoplasm of the egg cell causes reactivation of the genes responsible for sporophytic growth; the consequent messenger RNA's are already beginning to appear before fertilization. Support for this hypothesis comes from experiments in which egg cells are allowed to mature in the presence of uridine analogues.

The mature spermatozoid by contrast is little more than a nucleus provided with a motor apparatus. The chromatin is highly condensed, and it seems likely that transcription is dormant. Other than contributing a complement of Mendelian genes to the zygote, the main function of karyogamy may be to bring about the reorganization of the female nucleus. Decondensation of the male chromatin is accompanied by recondensation of the female, the opposed processes co-terminating in prophase of the first division.

With regard to the change from sporophyte to gametophyte, particular significance is seen in the thickened wall which surrounds the spore mother cell. Labelling experiments indicate that this is an effective barrier to complex molecules. Sporogenesis is envisaged as taking place in a confined and nutritionally deprived environment. The cytoplasm is further impoverished by the demands of the meiotic nucleus and is ultimately, with little opportunity for replenishment, shared between the four spores of the tetrad. Although haploid like that of the egg cell, the genome of the spore is supported by a depleted cytoplasm, unlike that of the egg cell. In these circumstances the activation of the sporophytic genes cannot be sustained, and the gametophytic are consequently expressed.

This interpretation of the significance of sporogenesis in the cycle receives support from the experimental investigation of apospory. Isolation from the correlative influences of the parent, together with subjecting the cells concerned to metabolic stress, restore the ability of the cell to divide but restrict the ensuing growth to gametophytic morphology.

Heterosporous cycles in the Pteridophyta can be reconciled with these views. In megasporogenesis the effects of meiosis are reversed by the particularly rich environment in which the surviving megaspore matures.

The strong sporophytic tendencies of the egg cells subsequently produced are indicated by the numerous records of parthenogenesis, a phenomenon of which there are no well-established instances in the homosporous Pteridophyta. The obligately apogamous ferns, with their larger spores and rapidly maturing gametophytes, occupy an intermediate position.

Резюме

Факторы регулирующие цикл развития папоротниковых (Pteridophyta): гаметофит/спорофит/гаметофит были заново изучены учитывая современные знания гаметогенеза, спорогенеза, апоспории и апогамии.

Ультраструктурные и цитохимические особенности гаметогенеза указывают на то, что оогенез является особенно важным в связи с переходом репродукционной фазы от гаметофита в спорофит. Яйцеклетка богата снабжена цитоплазмой и она созревает в закрытой камере (в архегонии или яйцевой мешочке), где имеется возможность усваивать вещества, образующихся в результате процесса лизиса других клеток в яйцеводе. Цитоплазма зрелой гаметы богато снабжена органеллами и рибосомами и содержит основные количества РНК и ДНК. ДНК в основном находится в органеллах но небольшое количество может присутствовать в свободной форме в цитоплазме связано с гистонами. Хроматиновые зёрна очень мелькие, не дают ощутимую реакцию Фелгена, хромосомы не обнаружимы. Предпологается что свойственное обогощение цитоплазмы яйцеклетки вызывает реактивацию генов, ответственных за спорофитический рост, а последующие мРНК-ы уже начинают появляться перед оплодотворением. Утверждением этого гипотеза могут служить результаты таких опытов, где яйцеклетки созревают в присутствии аналогов уридина.

Зрелый сперматозоид только на немного больше одного ядра снабженного моторным аппаратом. Хроматин высоко конденсирован и транскрипция кажется находиться в стадии покоя. Главная функция кариогамии может привести к реорганизации женского ядра и облегчить деление больше чем осуществлять менделевского расщепления генов в зиготе. Деконденсация мужского хроматина связана с реконденсацией женского и эти противоположные процессы содействуют в профазе первого деления.

В связи с переходом от стадии спорофита в гаметофит особое значение имеет утольщенная клеточная стена около материнской клетки споры. Опыты с мечением указывают на то, что она (кл. стена) и является мощной границей для комплексных молекул. Спорогенез очевидно происходит в ограниченных и бедно снабженных условиях. Цитоплазма ещё дальше беднеет в связи с потребностями мейотичес-

кого ядра и наконец без возможности обновления распределяется между 4 спорами тетрады. Геном споры как и яйцеклетки является гаплоидным но различается наличием обедненной цитоплазмы. В этих условиях активация спорофитических генов не может произойти и в следствии этого проявляются гаметофиты.

Такая интерпретация важности спорогенеза в цикле развития поддерживается экспериментальными исследованиями апоспории. Изолирование влияния родителя вместе с подвержением клеток метаболическому стрессу восстанавляет способность клетки к делению но ограничивает последующий рост гаметофитической морфологии.

Гетероспоровые циклы папоротниковых (Pteridophyta) могут быть пересмотрены учитывая этих взглядов. В мегаспорогенезе эффекты мейоза превращены необычайно богатыми условиями, в которых переживающая мегаспора созревает. Сильные спорофитические тенденции образующихся яйцеклеток поддержаются многочисленными случаями партеногенеза. Этот феномен неособенно характерен для гомоспоровых папоротниковых. Объязательно апогамовые папоротники со своими гораздо большими спорами и быстро созревающими гаметофитами занимают промежуточное положение.

II. Introduction

The existence of a free-living gametophyte in the Pteridophyta has long attracted the attention of those interested in the interpretation of the life cycle of the vascular plants. Fossil evidence continues to strengthen the view that the antecedents of our present land flora were archegoniate, and consequently an evolutionary element is added to the causality problems posed by the alternation of gametophytic and sporophytic phases in the cycle. In its simplest form the gametophyte/sporophyte cycle is in register with a haploid/diploid nuclear cycle, but it has long been clear that the number of sets of chromosomes in the nucleus is not in itself determinative of the kind of growth or reproductive behavior.

The times of transition from gametophyte to sporophyte and vice versa are also in the simplest form of cycle coincident with fertilization and sporogenesis. Again, however, stable changes of phase are possible without these events. Although a life cycle may appear normal morphologically, gametogenesis and sporogenesis may nevertheless occur only in a reduced or modified form.

The pteridophyte life cycle as we know it is the product of many millions of years of evolution, much of this evolution having been in the algal ancestors of the land plants. It is not surprising that selective forces have led to the economy of combining events of different significance in a single phenomenon. Sporogenesis, for example, combines meiosis, with its im-

portant function of providing an opportunity for genetic recombination, with the switch in gene activation which initiates the change in phase from sporophyte to gametophyte. Amongst the vascular plants the Pteridophyta alone have the advantage of providing situations, both natural and experimental, which facilitate the recognition of those factors primarily responsible for controlling the cycle. These situations can, however, be satisfactorily interpreted only against a background of current knowledge of gametogenesis and sporogenesis. It is consequently with these topics that this article begins.

III. Gametogenesis

A. FEMALE GAMETOGENESIS

In all Pteridophyta oogenesis takes place within the archegonium. Since not only the mature female gamete, but also the cell lineage which leads to it, have unique cytological features, it will be necessary to review the essential features of this characteristic organ.

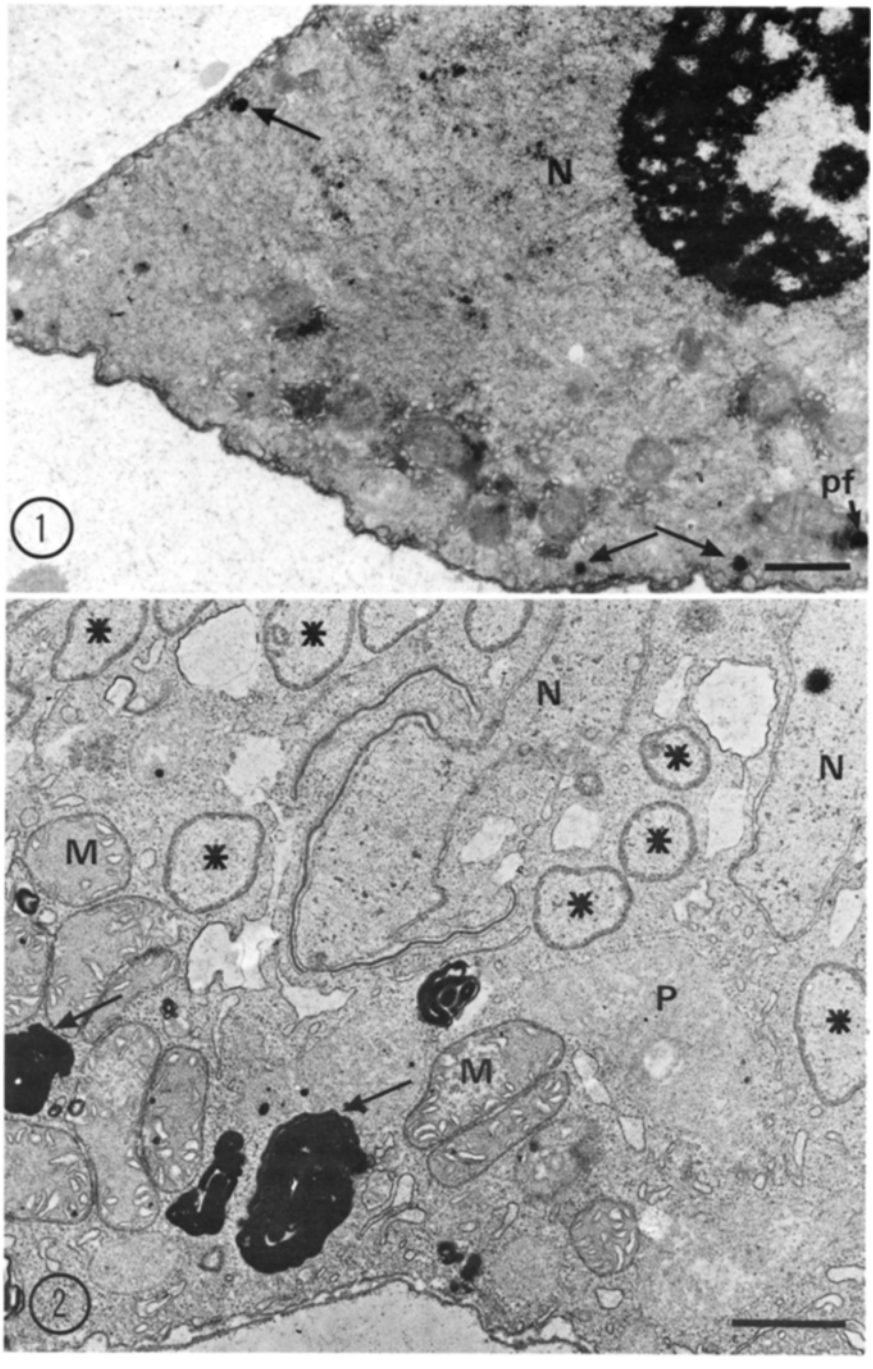
The structure of the archegonium is uniform to the extent that a neck, venter and egg chamber can always be recognized. The differences lie principally in the extent of the development of the neck, and the degree to which the archegonium is embedded in the parent gametophyte. The beginning of female gametogenesis seems always to consist of a periclinal division within a superficial cell of the gametophyte. The inner cell, either immediately or following a further periclinal division, yields the contents of the archegonial canal, namely the neck canal cells, and the single central canal cell and egg cell. The number of neck canal cells varies from one, in the archegonia of heterosporous pteridophytes, to many, as in some species of *Lycopodium.* The cell from which the contents of the canal are derived is termed the primary cell of the axial row.

Detailed studies of oogenesis have been carried out principally with homosporous ferns (Bell, 1979a), but it seems likely from the general resemblance between archegonia that the process is similar in essentials in all vascular archegoniates. The first striking feature, which can be regarded as marking the beginning of oogenesis, is the growth of the primary cell. In *Pteridium,* the species most studied, the volume of this cell increases by a factor of about five, and the nucleus, containing a prominent nucleolus, reaches a diameter approaching 10 μm. The large central vacuole disappears, and the cytoplasm becomes rich in ribosomes. There is also evidence of localized autophagy. Electron micrographs show regions of cytoplasm apparently being engulfed by small vacuoles, and also many vesicles containing recognizable fragments of organelles. These become particularly conspicuous as the nucleus of the primary cell enters mitosis (Bell, 1969). Not surprisingly, the growth of the primary cell and

the reorganization of its cytoplasm is accompanied by the active synthesis of RNA and protein (Cave & Bell, 1974a).

Division of the primary cell is strikingly unequal. Most of the cytoplasm remains at the base of the canal and becomes the cytoplasm of the central cell. This is indicative of strongly polarizing influences in the archegonial canal, and in *Marsilea* these can be effectively disturbed by chloral hydrate, a powerful mitotic poison (Kermarrec & Tourte, 1985). Quantitative autoradiography shows that the synthesis of RNA in the central cell remains high, and may even increase in the central cell, but the synthesis of protein begins to fall (Cave & Bell, 1974a). Cytologically, the principal change in the central cell is a notable increase in the number of vesicles, giving the cytoplasm at this stage a frothy appearance in the light microscope. These vesicles are associated with the enzyme acid phosphatase (Tourte, 1970), a reliable indicator of their autolytic function. Towards the end of the life of the central cell the vesicles, containing fragments of membranes and accumulations of osmiophilic material, begin to move to the periphery of the cytoplasm. The nucleus of the central cell has by this time become large and irregular in outline. The nucleolus remains conspicuous.

The next mitosis yields the egg and ventral canal cells. This division is also unequal and most of the cytoplasm remains with the egg. When it is first formed the egg cell is not capable of being fertilized. Not only is the archegonium unable to open, but the egg itself has first to go through a process of maturation. In leptosporangiate ferns this takes about 24 hours, and involves far-reaching changes in cytology and cytochemistry. In the first few hours of the life of the cell the vesicles containing osmiophilic material complete their movement to the periphery, and the material itself is secreted onto the surface of the egg (Cave & Bell, 1974b). In this way the egg cell acquires an extra envelope outside the plasmalemma (e.g., Fig. 1). Although acetolysis-resistant, this envelope does not consist of sporopollenin, but of a highly polymerized lipid, possibly resembling cutin (Cave & Bell, 1974b). The plastids become progressively more dedifferentiated and starch mostly disappears, but the mitochondria remain conspicuous. About 12 hours after the formation of the egg cell the chromatin in the nucleus becomes highly dispersed and ceases to respond detectably to Feulgen staining. In *Pteridium* and several other leptosporangiate ferns (Bell, 1979a; Bell & Duckett, 1976) the nucleus also becomes physically active at this time. Numerous protrusions, each bounded by both membranes of the nuclear envelope, begin to penetrate the cytoplasm (e.g., Fig. 2). The form of these protrusions depends upon the species. In *Pteridium* they are principally sac-like, with only a narrow channel, rarely exceeding 50 nm in width, maintaining connection with the main body of the nucleus. More complex forms also occur, but in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Pteridium they do not approach the intricacy of the convoluted and repeatedly invaginating protrusions found in *Dryopteris* (Bell, 1974a) and *Histiopteris* (Bell, 1980). These more complex protrusions frequently contain internal membrane systems.

A striking feature of the nucleus of the mature egg is the large nucleolus, often reaching as much as 6 μm in diameter, and frequently containing numerous lacunae in which fibrillar material is evident (Bell, 1970). Close to the nucleolus and elsewhere in the nucleoplasm are frequent nuclear bodies about 200 nm in diameter. These eventually find their way into the nuclear protrusions (Bell, 1972). Although they are usually smaller in the protrusions than in the main body of the nucleus they are wider than the connecting channel. Electron micrographs indicate that a protrusion forms as a nuclear body enters an embayment of the envelope, so pinching the nuclear body off from the main part of the nucleus (Bell, 1972). The membranes of the protrusions are sites of acyl transferase activity (Cave & Bell, 1975), suggesting that they are formed by localized growth of the envelope, and this may be stimulated by the approach of the nuclear body. There is no evidence that the bodies contain either DNA or RNA; they seem to consist entirely of acidic protein (Bell, 1983a).

As maturation of the *Pteridium* egg proceeds, the organelles become increasingly dedifferentiated. The plastids, large and amoeboid, possess a dense stroma, often including aggregates of phytoferritin (Sheffield & Bell, 1978; see also Fig. 1). The envelopes also lose their affinity for osmium, possibly a consequence of the lipids in the membranes becoming less unsaturated (Bell, 1983b; see also Fig. 2), and the membranes in consequence becoming increasingly permeable to metabolites. The mitochondria are often cup-shaped, the envelope of the cup region enclosing very little matrix and the villi mostly confined to the swollen rim. Conspicuous by their absence from the cytoplasm of the mature egg cells of *Pteridium* and *Dryopteris* are microtubules, although they are found in *Anemia* (Schraudolf, pers. comm.) and *Todea* (Bell, 1986). The truly remarkable feature of the cytoplasm is its richness in DNA. Grain counts after feeding with tritiated thymidine show that the extent of the incor-

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Fig. 1. *Pteris cretica.* Portion of the egg cell containing an irregularly shaped nucleus (N), with large conspicuous nucleolus. The plastids have started to dedifferentiate, and some contain phytoferritin (pf). Osmiophilic material can be seen in the peripheral cytoplasm (arrows) which may provide material for the layer external to the plasmalemma of the egg. Electron micrograph by F. A. El-Desouky. Bar = 1.5 μm.

Fig. 2. *Thelypteris palustris.* Portion of an egg cell showing a highly irregularly-shaped nucleus (N) with protrusions (*). M = mitochondrion; P = plastid, the boundaries of which are indistinct; arrows indicate osmiophilic material thought to be secreted onto the egg surface. Bar = 1 μm.

poration into the cytoplasm far exceeds that into the nucleus (Bell, 1960). The cytoplasmic labelling is largely a consequence of the amplification of the organellar DNA, but careful autoradiographic studies at high resolution have revealed that not all the DNA can be satisfactorily accounted for in this way (Sigee & Bell, 1971). Plasmids are known to occur in the nuclei of eukaryotic cells (Esser et al., 1986). It is therefore conceivable that the small amount of cytoplasmic radioactivity detectable in egg cells which it is not possible to relate to organelles may arise from short lengths or circles of DNA which leave the nucleus during maturation. It may be significant in this connection that, in contrast to a somatic cell, the cytoplasm of the egg cell is richer in basic proteins (as revealed by the Sakagouchi reaction) than the nucleus (Bell, 1963). Chromatin-like material may therefore be present in the cytoplasm in the mature egg cell. Bodies very similar in size and texture to the nuclear bodies are also found lying freely in the cytoplasm. Like the nuclear bodies, they lack RNA and probably consist entirely of acidic protein (Bell & Pennell, 1987). The evidence of the electron micrographs points to this material being secreted from the nuclear protrusions. There is no support for the view of Tourte (1975) that the nuclear protrusions break open and release material rich in ribonucleoprotein into the cytoplasm.

In *Pteridium* the egg is ready for fertilization about 24 hours after its formation. During this time the cytoplasm of the ventral canal cell shrinks to a structureless mass, and the nucleus becomes highly condensed. The contents of the binucleate neck canal cell by contrast undergo autolysis. Feulgen staining of half-mature archegonia shows that the nuclei of the neck canal cell become replaced by faintly staining material dispersing throughout the canal. The oligonucleotides generated by the autolysis of the DNA may move to sites of active polymerization in the maturing egg. The inner boundary of an archegonium at this time is markedly thickened, and in one instance at least has been shown to be callosed (Gorska-Brylass, 1968). It seems unlikely that large molecules liberated by autolysis would be able to diffuse out of the canal and be lost from the archegonium.

Although the nucleo-cytoplasmic interaction described for *Pteridium* has been encountered in the egg cells of other leptosporangiate ferns (Bell, 1979a, 1980), there are exceptions. Nuclear protrusions have not so far been detected in maturing eggs of *Pteris ensiformis* or *Ceratopteris* (Bell, 1984, 1986). Also, the egg cells of eusporangiate ferns are structurally very different from those of the eggs of the leptosporangiates. They are packed with large amyloplasts and nuclear protrusions appear to be absent (Bell, 1984, 1986). *Todea* has a similar egg cell, rich in amyloplasts, but here occasional narrow extensions of the nucleus have been detected (Bell, 1986). The egg cells of *Equisetum* are surprisingly like those of a eusporangiate fern, and similarly lack nuclear protrusions (Bell, 1970). There

appears as yet to be no detailed knowledge of oogenesis in *Lycopodium.* Oogenesis in heterosporous pteridophytes probably follows the same general course as in the homosporous, but only *Marsilea* (Tourte et al., 1971) and *Selaginella* (Robert, 1972) have so far been investigated ultrastructurally. In both the vesicular phase in the central cell appears to be absent, and there is no special envelope around the egg cell. In *Selaginella kraussiana* the mostly small mitochondria are confined to the upper part of the cytoplasm and curious cup-shaped plastids, with very little internal structure, are clustered around the nucleus (Robert, 1972). Nuclear protrusions have not been detected in the egg cell of any heterosporous pteridophyte.

B. MALE GAMETOGENESIS

The normal sexual cycle necessarily involves spermatogenesis. This is as remarkable cytologically as oogenesis, but wholly different in nature. Whereas the egg cell has a highly differentiated cytoplasm, in spermatogenesis, apart from the formation of the flagella (preceded by the appearance of centrioles, otherwise absent from the life cycle), it is the reorganization of the nucleus which is particularly notable.

Spermatogenesis occurs within antheridia, which, although variable in position and size within the Pteridophyta, are relatively simple organs. Each is a sac containing spermatocytes. In the leptosporangiate ferns these are commonly 32 in number, and are descended from a single primary spermatogenous cell. The situation in other pteridophytes, particularly those with large sunken antheridia (as in the lycopods) is not well known. Although there are cytologically interesting features in the differentiation of antheridia (e.g., Kotenko, 1985), these have no evident significance in relation to the life cycle. The development of the whole cell lineage is probably the same in essentials in all Pteridophyta.

In *Lycopodium,* which has biflagellate spermatozoids, the first visible sign of gamete differentiation is found within the spermatocyte mother cell. Two centrosomes are seen on opposite sides of the nucleus (Robbins & Carothers, 1975). It is possible that at an earlier stage, as in ferns, these arise from a single body adjacent to the nucleus. In any event the origin of these centrosomes, or their precursor, is clearly *de novo* since they are absent from preceding cell generations. The centrosomes at the earliest stage so far detected each consist of a co-axial pair of centrioles, joined base to base by a continuation of the central cartwheel portion. The alignment of the two centrioles is usually not quite perfect, a slight angular displacement occurring at the junction. Serial transverse sections of the centrosome show that the imbrication of the triplets reverses in passing from one centriole to its partner. This does not indicate any difference in

structure of the centrioles since, in the first centriole, the imbrication of the triplets is being viewed from its distal end (relative to the base of the centriole) and, in the second, from the proximal. Microtubules are frequently seen close to the centrosome, but they seem not to adopt any particular order or alignment. No information is yet available regarding the centrosome stage in *Selaginella.*

In those pteridophytes with multiflagellate spermatozoids the earliest stage of differentiation is represented by the blepharoplast. In the leptosporangiate ferns this first appears in the spermatocyte mother cell as a more or less spherical amorphous aggregate of osmiophilic material not far from the nucleus (Vaudois & Tourte, 1979). It divides before the nucleus enters prophase, and one daughter blepharoplast passes to the vicinity of each pole of the ensuing spindle. Each spermatocyte thus receives one blepharoplast, often, like the centrosomes of biflagellate spermatozoids, associated with irregularly arranged microtubules (Bell, 1974b). The situation in *Equisetum* is similar. In the heterosporous pteridophytes comparable information is available only from *Marsilea* (Hepler, 1976). Here a blepharoplast arises in the cell preceding the spermatocyte mother cell, but this degenerates at the onset of mitosis.

Subsequently a second blepharoplast arises close to the nucleus of the spermatocyte mother cell. This soon acquires a bipartite structure and subsequent development is as in the leptosporangiate ferns. No investigation of spermatogenesis in *Isoetes,* also heterosporous and with multiflagellate spermatozoids, has yet been completed at high resolution, although detailed observations were made by Yuasa (1938) at light microscope level. Knowledge of the initiation of the flagellar apparatus would be valuable since it might give an indication of whether the biflagellate condition was primitive or derived in the lycopod line. If the initial stages in *Isoetes* were found to be similar to those in the ferns, then *Isoetes* may have retained a feature of the earliest lycopods. Certainly the presence of multiflagellate spermatozoids in *Psilotum* and *Tmesipteris* (also awaiting investigation) suggests that the multiflagellate condition may be of considerable antiquity in the pteridophytes generally. If, on the other hand, the spermatocyte mother cell of *Isoetes* were found to contain multiple centrosomes it would seem more likely that the multiflagellate condition was derived, in line with the specialized nature of the plant as a whole, the spermatozoids of the earliest lycopods being biflagellate.

The first observable change in the spermatocyte itself is the further development of the centrosome or blepharoplast. In *Lycopodium,* for example, the centrosome divides transversely to form two centrioles which come to lie side by side on what is now recognizable as the anterior end of the cell (Carothers et al., 1975). These centrioles act as the basal bodies of the flagella. In *Pteridium,* by the time the spermatocyte mother cell has completed its division, the blepharoplast has reached a diameter of

about 0.5 μm. In the young spermatocyte the blepharoplast is seen to have acquired a tubular structure. The tubules, each about 50 nm in diameter, are radially arranged, and some appear to be continuous through the center. The tubules contain a core about 10 nm in diameter, from which ill-defined arms radiate (Bell & Duckett, 1976). The blepharoplast of *Marsilea* develops similarly, but it reaches a diameter of about 0.8 μm and has more tubules. Following the tubular phase the blepharoplast continues to grow and undergo further development. In *Pteridium* the diameter increases to about 1.5 μm, and the blepharoplast now consists of a central matrix of finely granular material surrounded by a shell of about 40 basal bodies, each about 0.5 μm in diameter and 0.3 μm in length. These show the characteristic structure of nine imbricating triplets (with singlets and doublets sometimes being seen in intermediate stages of development) with nine 'spokes' radiating to the triplets from the central 'hub.' Development is again similar in *Marsilea,* but during swelling to a diameter of about 1.25 μm the blepharoplast acquires a uniformly textured interior before the appearance of the basal bodies at the periphery.

From this point on rapid and extensive changes take place which transform the spermatocyte into the motile gamete. The differentiation of the motile apparatus starts with the appearance of the multilayered structure (MLS). In *Lycopodium* this develops beneath the two basal bodies at the anterior end of the spermatocyte. Two components are visible, the lower consisting of a series of vertical plates arranged parallel to each other, and the upper of microtubules (Robbins & Carothers, 1978). The microtubules lie parallel in a single plane, and are linked to each other by cross bridges. Within the lower (lamellar) portion of the MLS three layers can be distinguished. The upper two are separated by a line of demarcation formed by small masses of opaque material between the plates. In the third lowermost layer the image of the plates is much less sharp. The thickness of the whole lamellar strip is about 6 nm. The MLS lies above a particularly conspicuous mitochondrion. In *Selaginella* the two centrioles move further apart, one remaining anterior and the other becoming clearly posterior to it. The MLS and its associated mitochondrion appear between the centrioles as they take on the function of basal bodies (Robert, 1974).

In those Pteridophyta with multiflagellate spermatozoids the MLS arises in a very rudimentary form within the shell of basal bodies forming the periphery of the differentiated blepharoplast. As the blepharoplast opens the MLS moves away from the basal bodies and associates with the mitochondrion (Fig. 3). Subsequently the MLS and the mitochondrion grow in step with each other, the former by increase in number of both plates and microtubules. Even at the first appearance of the MLS it is possible to see that there is an angle of about 45° between the alignment of the plates and that of the microtubules. This divergence is maintained in all subsequent development.

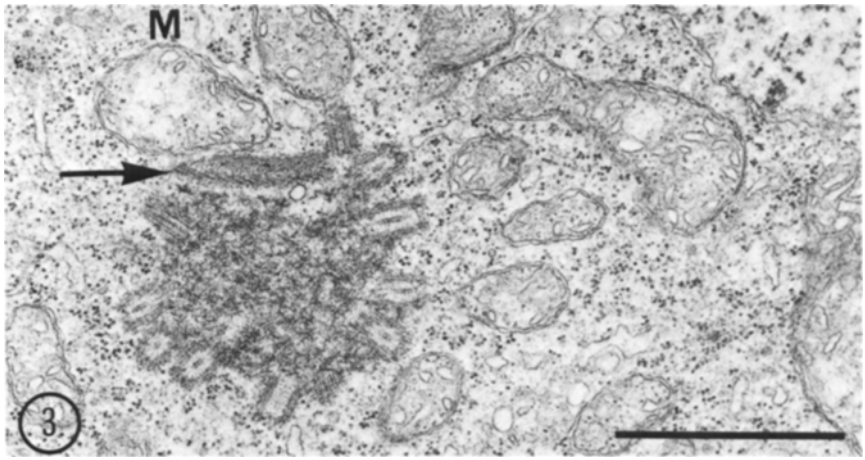


Fig. 3. *Pteridium aquilinum.* Blepharoplast in a young spermatocyte. The multilayered structure (arrow) is emerging from the blepharoplast and associating with a mitochondrion (M). The outer part of the blepharoplast has differentiated into basal bodies. Bar = 1 μm.

Following the establishment of the MLS-mitochondrion complex the differentiation of biflagellate and multiflagellate spermatozoids is basically similar. The upper layer of the MLS extends posteriorly as a ribbon of microtubules. Simultaneously, the region of the nucleus closest to the posterior edge of the lamellar strip rises as a narrow crest, its summit running parallel to the side of the strip and along the whole of its length. In *Pteridium* there are indications that material for assembly of the lamellae may leave the perinuclear space by way of blebs at the tip of this nuclear crest (Bell & Duckett, 1976). The proximity of the crest and the MLS is such that the extending ribbon of microtubules grows over the nuclear surface, although a clear space about 20 nm in width remains between the ribbon and the outer membrane of the nuclear envelope. The envelope beneath the ribbon seems to lack nuclear pores, a feature elegantly demonstrated in *Marsilea* by freeze-fracture (Myles et al., 1978). As the MLS as a whole grows it describes a left-handed helix (when viewed from the anterior), extending at maturity for distances ranging from about one gyre (*Marsilea*; Myles & Bell, 1975) to fractionally more than two gyres (*Equisetum*; Duckett & Bell, 1977). Extension of the microtubular ribbon is accompanied by movement of the basal bodies towards their final positions. In *Lycopodium* the two basal bodies are now found on opposite sides of the nucleus, close to the microtubular ribbon and oriented parallel to it (Robbins & Carothers, 1978), the distal end of the basal body being directed posteriorly. In multiflagellate spermatozoids the basal bodies begin to be distributed, together with osmiophilic material

similar to that originally at the center of the blepharoplast, along the microtubular ribbon (Bell & Duckett, 1976; Myles & Hepler, 1977).

During extension of the microtubular ribbon extensive changes take place in the nucleus. The chromatin begins to condense and the nucleoplasm is progressively eliminated. In some forms, such as *Selaginella* (Robert, 1974) and *Marsilea* (Myles & Hepler, 1977), longitudinal strands are clearly recognizable in the condensing chromatin, but similar elements have not been detected in *Equisetum* and *Pteridium.* While the chromatin is condensing the nucleus is also changing its shape, but the extent of the change varies very much with species. In *Lycopodium* the nucleus remains more or less ovoid (Robbins & Carothers, 1978), but in *Selaginella* it is elongated. In *Equisetum* the nucleus falls into a weak helix of about 1.25 gyres (Duckett & Bell, 1977), while in ferns the sperm nucleus is longer and narrower, and extends for about three gyres of the helix in *Pteridium* (Bell & Duckett, 1976) and about four gyres in *Marsilea* (Myles & Bell, 1975). Whatever the shape of the nucleus the microtubular ribbon runs over its surface, and in the helicoid spermatozoids of the ferns the elongated nucleus comes into register with the helix of the microtubules.

In the final stages of differentiation the MLS tends to move away from the nucleus. In consequence the nucleus is usually absent from the anterior gyre of the helix. The chromatin now becomes completely condensed and often (as in *Equisetum*) encloses particularly densely staining nodules, each surrounded by a narrow translucent zone (Duckett & Bell, 1977). The behavior of the nuclear envelope is variable. In *Equisetum* and *Lycopodium* it is visible in the mature gamete, but in *Pteridium* and *Scolopendrium* the perinuclear space is eliminated and the membranes of the envelope come together and are closely applied to the surface of the chromatin. The microtubular ribbon in its final stages becomes tightly bound to the condensed nuclear envelope (Bell, 1978; Robert et al., 1986). In *Marsilea* the nuclear envelope is eliminated and the surface of the chromatin is naked (Myles & Bell, 1975). The cytoplasmic organelles, apart from the several mitochondria associated with the MLS, tend to be confined to the posterior portion of the gamete. The cytoplasm containing them is either retained, as in *Lycopodium,* or is confined to a terminal vesicle and shed during swimming, as in *Pteridium* and *Marsilea.* As a consequence in the ferns, despite earlier reports (probably arising from misidentification of badly fixed organelles), it is doubtful whether male plastids ever enter the egg. The growth of the flagella is very rapid and is probably completed before the final differentiation of the body of the gamete. The lamellar strip of the MLS remains clearly visible in mature spermatozoids of *Lycopodium, Equisetum, Pteridium* and *Marsilea,* but can no longer be discerned in *Selaginella.* In this, and in its almost linear form, the spermatozoid of *Selaginella* resembles that of many bryophytes

(Robert, 1974). Surveying the male gametes of the Pteridophyta as a whole (so far as they are known), the spermatozoid of *Lycopodium* (Fig. 4), where the helical MLS hardly affects the generally ovoid shape of the cell, seems to be the least specialized. At the other extreme is the spermatozoid of *Marsilea* where the whole cell (except for the discarded cytoplasm) is transformed into a long narrow helix of up to ten gyres. The spermatozoid of *Pteridium,* with about five gyres, occupies an intermediate position.

The male gamete of the Pteridophyta is a cell unique in the life cycle. At what stage is the information for this unique differentiation transcribed and translated? The visible differentiation is accompanied by condensing chromatin, not a condition normally associated with active transcription. This suggests that differentiation begins cytochemically before it becomes detectable visually. This view is strongly supported by work with *Marsilea.* The abundance of microtubules present in the differentiated spermatozoids, in both the microtubular ribbon and flagella, indicates that a protein present in substantial quantity will be tubulin. Gel electrophoresis has shown that tubulin increases very strikingly up to the time at which the blepharoplast appears and differentiation begins, but that subsequently the increase is negligible (Hyams et al., 1983). The final stage of differentiation of the spermatozoid must therefore await a signal which initiates the assembly of tubulin already present in the cells. The nature of this signal, and the manner of control of the subsequent assembly are so far unknown.

In those pteridophytes with helical spermatozoids the manner in which the extending microtubular ribbon is caused to follow a helical path presents intriguing geometrical problems. The MLS, which appears to contain unpolymerized tubulin (Wolniak, 1983), is probably an elaborate microtubule organizing center. Since the lamellar strip is itself curved, it seems likely that the ribbon grows from this site (current studies of microtubule polarity are likely to answer this point). The microtubules would therefore be assembled on a curved surface. If the microtubules retain their curvature (which, because of the angular displacement between the alignments of the plates and of the tubules, will be about half that of the lamellar strip) a helix will result as the ribbon extends. It is difficult, however, to see how microtubules could retain curvature unless they are in some manner held in the curved state. The explanation may lie in microtubule-associated proteins (MAP's). If, for example, the cross bridges which are conspicuous between the microtubules of the ribbon were slightly out of register, these might suffice to stabilize curvature in the emerging ribbon. The persistence of the helix of microtubules even after the spermatozoid is engulfed in the female cytoplasm and the sperm nucleus has fallen away from it (Bell, 1975) shows that the curvature of the ribbon is firmly implanted, and that it forms an effective cytoskeleton.

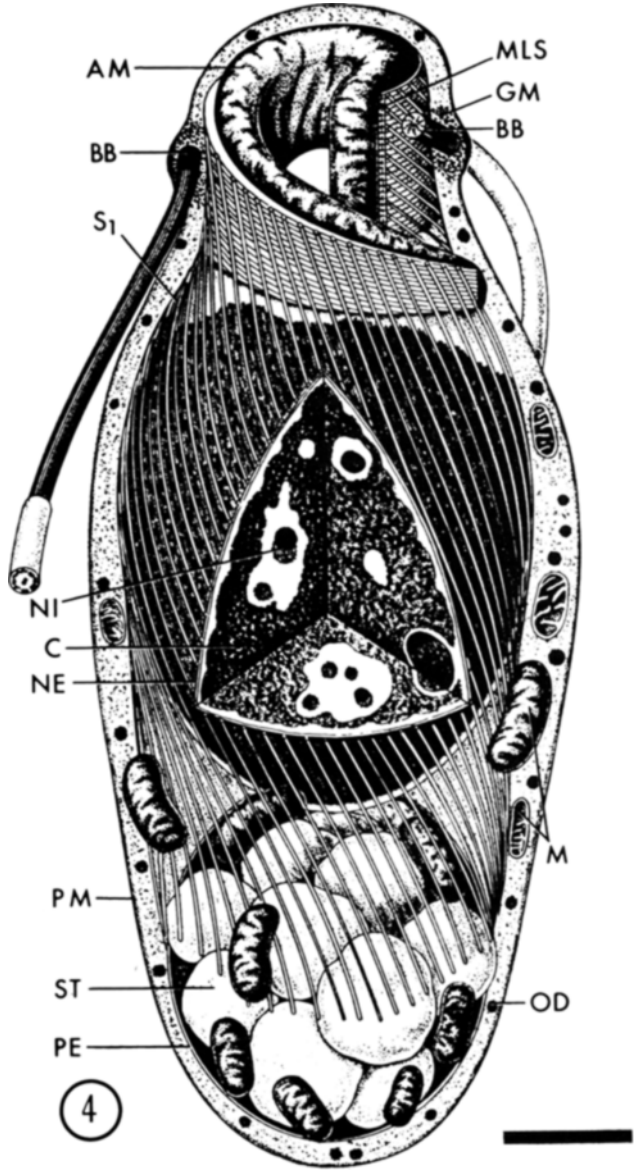


Fig. 4. *Lycopodium* (*Lycopodiella*) *cernuum.* Reconstruction of the mature spermatozoid. AM, anterior mitochondrion; BB, basal body; C, condensed chromatin; GM, globular matrix; M, mitochondrion; MLS, multilayered structure; NE, nuclear envelope; NI, nuclear inclusion; OD, osmiophilic droplet; PE, plastid envelope; PM, plasma membrane; S_1, microtubular ribbon (about every third microtubule illustrated); ST, starch. From Robbins & Carothers (1978). Bar = 1 μm (approx.). Reproduced with kind permission of the American Journal of Botany.

The use of anti-tubulins complexed with fluorochromes has proved useful in locating the blepharoplast in differentiating spermatocytes (Doonan et al., 1986; Mizuno et al., 1985). In *Pteridium* the blepharoplast found near each pole of the spindle at metaphase in the spermatocyte mother cell fluoresces when mounts of the whole cell are subjected to this technique (Marc & Gunning, 1986). Compared with the spindle, however, the fluorescence is very faint, and it would be hazardous to conclude that the undifferentiated blepharoplast contains tubulin. The fluorescence may in fact come from randomly scattered microtubules at the surface of the undifferentiated blepharoplast (Bell, 1974b). It is significant that the fluorescence at this stage is distinctly brighter at the perimeter of the 'blepharoplast' (its precise limits are impossible to determine in the light microscope) than at its center, an image quite different from that obtained subsequently (Marc & Gunning, 1986). This would be in line with its source being a superficial investment, and not an integral component of the spherical body. Using gold-labelled anti-tubulins and the superior resolution of the electron microscope Pennell et al. (1986) were able to show that in *Marsilea* the blepharoplast does not bind anti-tubulins until basal bodies begin to differentiate. Tubulin is not therefore a structural component of the undifferentiated blepharoplast, although it is conceivable that blepharoplast contains unpolymerized tubulin prevented by some form of masking from binding the antibody. Further cytochemical tests (Pennell et al., in prep.) have shown that the blepharoplast contains neither DNA nor RNA. It seems very likely that the blepharoplast consists wholly of protein, and prominent in its composition may be MAP's ultimately concerned with the assembly of the numerous microtubules of the motor apparatus. The preparation of a blepharoplast-rich fraction from immature spermatocytes, which would permit a more detailed biochemical analysis, has so far proved impossible.

Experiments with colchicine have shown that the elongation and coiling of the nucleus of the helicoid spermatozoids of ferns is dependent upon the growth of the microtubular ribbon (Cave & Bell, 1979; Myles & Hepler, 1982). It is not, however, simply a question of the extending ribbon exerting traction on the nuclear envelope, since there is regularly a space between the two. This may of course be illusory, since proteins with mechanical properties may be present at this site, but fail to be visualized by standard methods of preparation. Evidence that there is some factor which causes the movement of the chromatin towards that part of the nucleus subtended by the microtubular ribbon is provided by experiments with colchicine. In those instances where the ribbon develops as a number of strap-like fragments, condensed chromatin is found only in those parts of the nucleus where a fragment of ribbon is close to the envelope (Cave & Bell, 1979; Myles & Hepler, 1982). It does not seem

that actin is an intermediary. Although present in spermatozoids, actin does not become detectable until after nuclear shaping has begun, and it is initially restricted to the MLS (Marc & Gunning, 1986).

The cytochemistry of the sperm nucleus clearly demands further study. Protamine, the protein characteristic of vertebrate sperm, appears to be absent from pteridophyte sperm nuclei. In *Equisetum arvense* the DNA is said to be confined to the central region of the nucleus, associated with arginine-rich histone, non-histone proteins being aggregated locally against the envelope (Mabrouk, 1978). Polyacrylamide gel electrophoresis of the proteins present in acid extracts of the nucleus reveals two fractions. The first has a mobility of the same order as that of calf thymus histones, and the second a mobility intermediate between that of typical histones and protamine from salmon sperm (Mabrouk et al., 1979). The first fraction is also recoverable from the nuclei of young sporophytes. Similar results have been obtained with *Scolopendrium* spermatozoids (Robert et al., 1986). Those basic proteins responding to the ammoniacal silver reaction are confined to the outer part of the nucleus. The manner in which the chromatin is packed in sperm nuclei is also little known. At light microscope level the nucleus of the spermatozoid of *Pteridium aquilinum* can be made to break by fluid turbulence into a series of paired rod-like fragments, variable in number, but in about the same frequency as half the accepted haploid chromosome number ($n = 52$) (Wakeford & Bell, 1980). This result was of particular interest in view of the reports of a form of *Pteridium* (named *P. herediae* by Löve & Kjellqvist, 1972) with a haploid chromosome number of 26. The paired fragments in Wakeford's preparations, if *Pteridium aquilinum* were an autotetraploid derived from *P. herediae,* could therefore represent homoeologous chromosomes. Evidence for this view is, however, diminished by the lack of confirmatory chromosome counts for material recently collected at the Löve and Kjellqvist site (Sheffield et al., in prep.) and the finding that *P. aquilinum* behaves electrophoretically as a diploid (Sheffield et al., 1986; Wolf et al., 1987). Nevertheless, the tendency of the sperm nucleus to fall into rod-like fragments is shown also by *Marsilea.* Treatment of egg cells with colchicine at the time of fertilization is followed by the failure of karyogamy. The male nucleus, lying in the female cytoplasm, is then seen to split longitudinally, and each portion to fragment in the manner seen in *Pteridium* (Kuligowski-Andres et al., 1982; Kuligowski-Andres et al., 1985). Although the evidence from both *Pteridium* and *Marsilea* is not conclusive, it is possible that the chromosomes enter into the sperm nucleus in a regular linear order. This seems to be the situation in the sperm nucleus of the liverwort *Sphaerocarpus* (Reitberger, 1964). In many, and probably all pteridophyte spermatozoids with elongated nuclei, the chromatin is birefringent in the same manner as parallel strands of DNA, but a cor-

responding alignment is rarely directly visible in the nuclei of mature sperm. In the nucleus of the *Selaginella* spermatozoid extraction experiments indicate that the DNA is present in the form of narrow cylinders, about 60 nm in diameter, the center of each cylinder being occupied by non-histone proteins (Robert, 1977). Electron microscopy of the relaxed chromatin of the *Pteridium* spermatozoid also indicates the presence of cylinders (Wakeford, unpubl.). These are about 120 nm in diameter, but their identity with chromatin has yet to be demonstrated conclusively.

IV. Fertilization and Formation of the Zygote

Until relatively recently the ability of gametophytes of homosporous pteridophytes to produce both male and female sex organs had led to the supposition that they were highly inbred. Electrophoretic evidence is increasingly revealing, however, that outcrossing is the rule for many species (see Haufler, 1987, for review) and that even species such as *Pteridium* in which large stands often spore infrequently in nature reproduce predominantly by intergametophytic mating (Sheffield et al., 1986; Wolf, 1986). Comparatively little is yet known about the process of fertilization in the Pteridophyta, however. Archegonia are probably caused to open by the hydration of mucilage in the upper part of the canal, the upper tier of neck cells often being widely reflexed in consequence. Significant in this connection is that when gametophytes are grown in liquid culture, conditions in which the cells presumably remain uniformly hydrated, archegonia rarely open. The mucilage which emerges when an archegonium does open, gradually disperses. Careful observations in *Pteridium* show that two bodies are then ejected from the canal, probably derived from the remains of the ventral canal cell. A clear passage, possibly filled with a less viscous mucilage, then leads to the egg. The surface of the egg in the fully hydrated condition may be convex, instead of concave as usually seen in fixed material.

Chemotactic attraction of spermatozoids to newly opened archegonia may be a general phenomenon. The spermatozoids of many homosporous ferns will swim towards a source of malic acid, but it is not known whether this is a naturally occurring attractant. It seems likely that the chemotactic mechanism is not the same in all instances. The archegonial mucilage of *Dryopteris,* for example, has only a weak influence on the spermatozoids of *Athyrium.* Intergeneric incompatibility has also been claimed, the spermatozoids of one species being immobilized in the archegonial mucilage of the other (Schneller, 1981). These results have, however, to be treated with caution since it has been observed that the mucilage which first issues from the archegonium of *Pteridium* will immobilize spermatozoids of the same species. Only those spermatozoids arriving after this first mucilage

has cleared are able to enter the canal. The existence of intraspecific incompatibility remains disputed. The failure of selfed gametophytes to produce embryos may also result from an accumulation of lethals (Klekowski, 1979) or, in the case of species where the antheridial and archegonial phases are normally separated, from the non-viability of eggs produced in the presence of antheridia containing differentiated spermatocytes.

The diameter of the archegonial canal is usually less than that of the swimming spermatozoid. Consequently the gamete becomes elongated and narrower as it enters the canal and moves towards the egg by means of writhing movements. When it reaches the chamber above the upper surface of the egg the gamete regains its original form, providing a clear indication of the structural integrity of the helix. It seems likely that insemination is effective only if the anterior end of the spermatozoid makes contact with the egg. Although the evidence is far from conclusive it is possible that some of the flagella in this region have specialized regions at their tips which, by analogy with the pairing of *Chlamydomonas* gametes, bring about the fusion of the spermatozoid with the surface of the egg cell (Bordonneau et al., 1984). Electron micrographs of this stage are rare, but the situation shortly after contact has been clearly pictured in *Marsilea* (Myles, 1978). The female cytoplasm evidently rapidly ascends into the empty central part of the helix, the gamete being at the same time drawn into the egg. The initial stages of plasmogamy, with the formation of a transient fertilization cone, thus resemble those seen in many animals.

The subsequent events have been established in *Pteridium* (Bell, 1975). Although wholly engulfed in the female cytoplasm the microtubular ribbon retains its helical form, and the multilayered structure remains identifiable close to the female nucleus for at least six hours following syngamy. The nuclear envelope of the male nucleus becomes reconstituted, a perinuclear space reappears between the two membranes, and the outer membrane comes away from the microtubular ribbon. Relaxation of the male chromatin begins very soon after the spermatozoid enters the egg. In *Marsilea* at this stage the longitudinal strands seen during condensation once again become evident (Fig. 5). The envelope at the anterior end of the male nucleus soon makes contact with that of the egg nucleus, leading to the formation of a number of pores which expand and coalesce. The nucleus, now fully detached from the microtubular ribbon, collapses into an irregular shape. When the opening between the male and female nuclei is fully formed the male chromatin begins to flow into the female, the nuclear envelope of the male nucleus concomitantly reducing its area. Karyogamy in *Pteridium* is probably completed within 60 minutes of plasmogamy.

Although several spermatozoids are occasionally found within necrotic

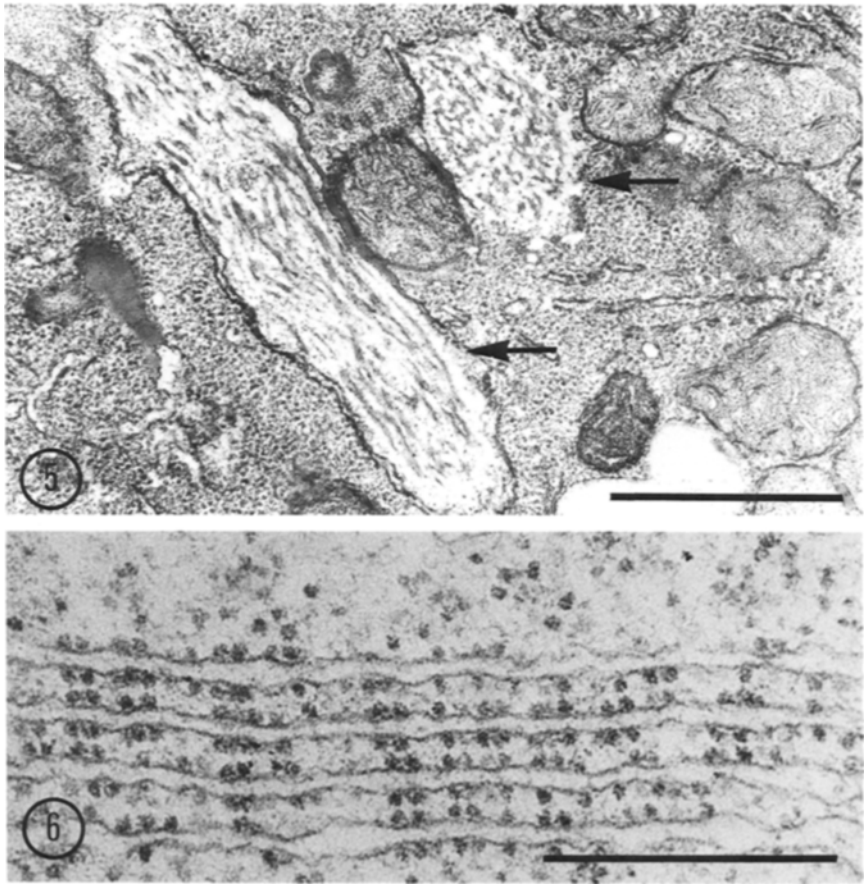


Fig. 5. *Marsilea vestita.* Sections of the helical nucleus (arrows) within the cytoplasm of the egg following fertilization. The chromatin has relaxed into rodlets similar to those seen during condensation of the chromatin in spermatogenesis. Electron micrograph by D. Myles. Bar = 1 μm.

Fig. 6. *Pteridium aquilinum.* Stacked endoplasmic reticulum and ribosomes in regular formation in an egg cell about 30 minutes after syngamy. Bar = 0.5 μm.

eggs of *Pteridium,* there is no evidence that polyspermy normally occurs, although excluded spermatozoids are often pressed close against the plasmalemma of the egg. It is not yet known how polyspermy is prevented. Presumably, if the initial recognition and union depend on the formation of a glycoprotein complex, as has been demonstrated in *Fucus,* the egg surface is irreversibly altered by the entry of the first spermatozoid, and no further acceptance is possible. Even if plasmogamy is successful, kar-

yogamy does not necessarily follow. Studies of *Pteridium* have shown that if the direction of entry of the spermatozoid is oblique, instead of perpendicular to the surface of the egg, the male nucleus may pass to one side of the egg nucleus and fusion fails to occur (Bell, 1975). This may be one explanation of the relative infertility of *Pteridium* gametophytes. Despite many archegonia often being inseminated when cultures of gametophytes are flooded, the gametophytes rarely produce more than one sporophyte. Experiments by Sheffield (1984b) have, however, shown that several sporophytes regularly arise from a single gametophyte after flooding if dimethyl sulphoxide (DMSO) is present in the substrate. Either, therefore, mature eggs remain viable longer in the presence of DMSO, or hormonal and nutritional interaction between zygotes is suppressed so that several embryos are able to become established. Polyembryony in these conditions is also accompanied by continued growth of the gametophyte, normally diminished following successful fertilization, again indicative of reduced correlative influences. Details of fertilization in Pteridophyta other than the ferns are largely unknown.

The formation and maturation of the zygote in the Pteridophyta has so far been little studied by modern techniques. In *Pteridium* shortly after fertilization stacks of endoplasmic reticulum with regularly arranged ribosomes are sometimes conspicuous (Fig. 6). Subsequently there is marked contraction of the zygote, as in the flowering plant *Gossypium* (Jensen, 1968). The fate of the mitochondria brought in by the male gamete is uncertain. In *Pteridium* these mitochondria, which are readily recognizable by their regular shape and well developed internal lamellae, become surrounded by endoplasmic reticulum and are possibly resorbed. The single large mitochondrion of the *Marsilea* spermatozoid (Myles & Bell, 1975) was said by Faivre et al. (1982), on the basis of inadequate evidence, to degenerate shortly after syngamy. In *Pteridium* nuclear protrusions present at the time of fertilization also become surrounded by endoplasmic reticulum, but their fate is uncertain. By the time the zygote nucleus moves into prophase (about 48 hours after fertilization) nuclear protrusions are no longer evident. Using tritiated thymidine as a tracer Faivre et al. (1982) have obtained evidence that in the zygote of *Marsilea vestita* the replication of the DNA contributed by the male gamete begins earlier than that of the female. Following the subsequent distribution of the radioactivity led Tourte et al. (1980) to the view that, although the maternal radioactivity is uniformly distributed in subsequent cell generations, that of the male becomes over-represented in the nuclei of those cell lines which will ultimately give rise to the organs of the embryo. This result is difficult to explain on the assumption of random segregation of daughter chromosomes at mitosis, and clearly needs further study.

V. Sporogenesis

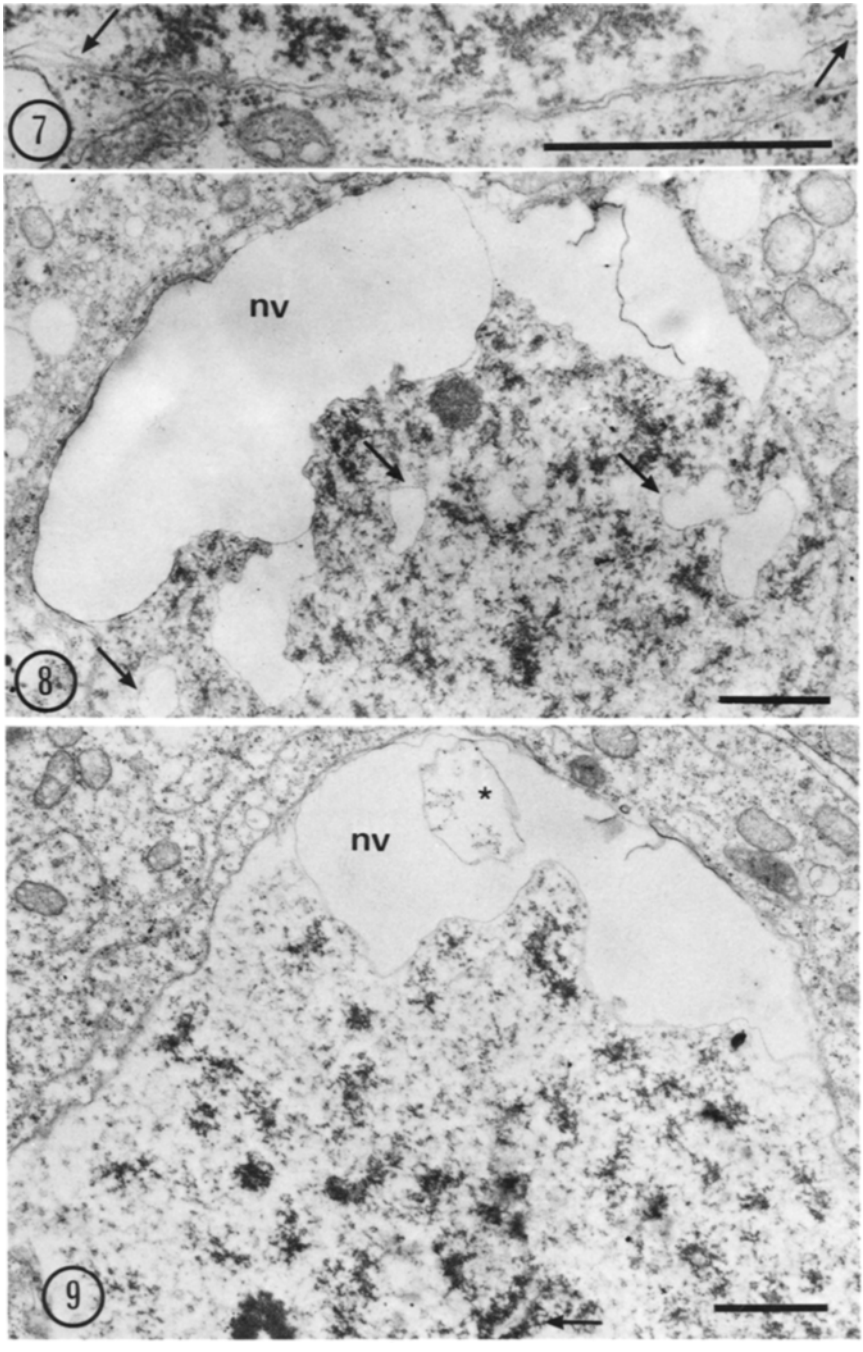
A. HOMOSPOROUS SPOROGENESIS

The light microscopic features of sporangial development and spore formation are well known, and have been reviewed by Marengo (1949) and Manton (1950). Ultrastructural events accompanying sporogenesis are less familiar and the process has been followed in detail for only two sexually reproducing pteridophytes, *Pteridium* (Sheffield & Bell, 1979) and *Psilotum* (Gabarajeva, 1984a, 1984b, 1985), although some data are available for *Onoclea* (e.g., Marengo, 1977), *Lycopodium* (Pettitt, 1978), *Anemia* (Schraudolf, 1984) and *Equisetum* (Lehmann et al., 1984; Bednara et al., 1986). From these studies it has emerged that the process resembles both microsporogenesis in angiosperms (e.g., Dickinson & Heslop Harrison, 1977) and sporogenesis in mosses (e.g., Neidhart, 1975; Brown & Lemmon, 1982). As in other land plants, the most striking subcellular changes occur during the formation and maturation of spore mother cells, cells which become progressively isolated from the surrounding tissue. At the onset of prophase of meiosis the sporogenous cells are interconnected by means of plasmodesmata, but during leptotene cytoplasmic connections between meiocytes and tapetal cells are eliminated. Fern spore mother cells remain in contact with each other via connections, which widen into large channels up to 0.6 μm in diameter. Such cytomictic channels serve to maintain synchrony between the sporogenous cells. The situation is similar in *Psilotum*, but here the tapetum subsequently intrudes between the spore mother cells, and their development then becomes asynchronous (Gabarajeva, 1984a). A thick wall develops around each spore mother cell at the onset of meiosis. Comparison with bryophyte and angiosperm studies would suggest that this wall is callosic in nature. Bienfait and Waterkeyn (1976), and Sheffield and Bell (1979), however, failed to obtain a positive reaction for callose in the walls of eusporangiate and leptosporangiate fern meiocytes with the Aniline Blue fluorescence staining method. It may therefore be that callose does not enter into the composition of this wall or that, as suggested by Smith and McCully (1978), secondary plant substances quench the fluorescence. The presence of considerable amounts of tannins and phenolics in ferns such as *Pteridium* (Cooper-Driver, 1976) could therefore account for the lack of a positive reaction for callose with the Aniline Blue technique. As each meiocyte progresses through prophase the ground cytoplasm loses much of its substance. The disappearance of ribosomes is accompanied by an increase in the number of vesicular profiles, probably indicative of widespread lysosomal activity. Simultaneously, the mitochondria and plastids disperse from around the nucleus and lose much of their internal lamellar structure. The prophase nucleus also undergoes

marked modification. Numerous vacuoles are formed by the invagination of the inner membrane of the envelope into the karyoplasm (Fig. 7; and see also Marengo, 1979, fig. 5). These vacuoles resemble those found in other plants and animals at this stage (Sheffield et al., 1979) and become progressively larger during prophase (Fig. 8). By the time synaptonemal complexes have formed, vacuolar profiles containing material similar in appearance to cytoplasm can be found throughout the nucleus (Fig. 9). Although cytochemical tests have proved inconclusive, it has been assumed that such vacuoles in angiosperms are autophagic and that they have engulfed elements of the cytoplasm (Sangwan, 1986). As pteridophyte nuclei remain essentially smooth and rounded throughout prophase it is difficult to imagine how this might occur, but these structures clearly merit further investigation.

The cytoplasm reaches its most extreme state of depletion during metaphase, then subsequently recovers. Young spores, therefore, although less well endowed than archaesporial cells, are nevertheless furnished with a full complement of redifferentiated organelles, and with ribosomes almost as numerous as those of pre-meiotic cells. Cutinization of the sporangial wall and deposition of exine render fixation and embedding increasingly difficult, so little is known of the maturing fern spore before its release. The appearance of dormant and newly germinated spores (see, for example, Bell, 1970; Marengo, 1973) indicates, however, that no major ultrastructural changes occur after spores separate from the tetrad.

In all the foregoing respects sporogenesis in pteridophytes conforms with that of land plants generally, but there are several other features which are dissimilar and worthy of mention. The most striking of these are changes which have been observed in the boundaries of the plastids of spore mother cells. During meiotic prophase in *Pteridium* (and *Dryopteris filix-mas*; Bell, unpubl.) the plastids pass through a stage during which no envelope can be resolved following aldehyde fixation, osmication and conventional staining techniques. This is the time at which the plastids in all instances of sporogenesis so far studied pass through a period of dedifferentiation, but only in a number of ferns does the envelope apparently vanish. As already discussed in relation to plastids in the egg cells of leptosporangiate ferns, whose boundaries behave similarly, the change in affinity for osmium tetroxide may indicate reduced unsaturation of the membrane lipids leading to increased permeability and enhanced metabolic interchange. Although the plastid envelopes become transiently invisible in a like manner during megasporogenesis in *Marsilea* (Bell, 1981), such changes have not been observed during sporogenesis in *Anemia* (Schraudolf, 1985) or in *Lycopodium* (Pettitt, 1978). Modification of the plastid envelopes during meiosis cannot therefore be an essential feature of sporogenesis in the Pteridophyta.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No elimination of plastids has been detected during sporogenesis in ferns, but in *Lycopodium* sheathing endoplasmic reticulum does appear to bring about the autolysis of a proportion of these organelles during meiotic prophase (Pettitt, 1978). Endoplasmic reticulum is also associated with plastids throughout sporogenesis in *Pteridium* and *Psilotum* but there are no indications that it is involved with autolytic activity. The association tends to become less close as the nucleus enters meiosis. During the first division of meiosis some plastids are entirely free from endoplasmic reticulum and others, frequently complex in form, are only loosely associated with single sheets of reticulum. The significance of this pleomorphy has yet to be determined but it does not appear to be linked with any elimination of plastids. Myelin figures, similar to those thought to represent degenerate plastids in *Lycopodium* (Pettitt, 1978), are found in association with plastids in *Psilotum* (e.g., Gabarajeva, 1984b) and infrequently in spores of *Pteridium* (Fig. 10), but their significance and origins are obscure. Despite the very rudimentary nature of plastids in spore mother cells of ferns there is no evidence that any fail to redifferentiate after meiosis.

In pteridophytes generally there are strong indications that grouping and apportionment of both plastids and mitochondria during meiosis and subsequently in sporogenesis are tightly controlled. At mid-prophase in spore mother cells of *Pteridium* (Sheffield, 1978) and *Equisetum* (Bednara et al., 1986) the organelles are aggregated into a single group. This has been observed in one cycad and some angiosperms (Rodkiewicz et al., 1986) but its significance is unclear. The grouping and close association of organelles in *Selaginella* at this time have been linked with the presence of special microtubules (Brown & Lemmon, 1985). The organellar plate which bisects the diads of ferns (e.g., Bell, 1981; Sheffield & Bell, 1979) is also found in *Psilotum* (Gabarajeva, 1985), *Equisetum* (Bednara et al., 1986) and some higher plants (Rodkiewicz et al., 1986). Instead of ensuring equal apportionment of organelles amongst the post-meiotic cells, Bednara et al. (1986) suggest that the plate may be more significant in preventing the interference of adjacent spindles in the second division of meiosis.

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Fig. 7. *Pteridium aquilinum.* Nuclear envelope at the start of meiotic prophase, arrows indicate invaginations of the inner membrane. Bar = 1 μm.

Fig. 8. *Pteridium aquilinum.* Portion of spore mother cell later in prophase where nuclear vacuoles (nv) can be seen throughout the karyoplasm (arrows). Bar = 1 μm.

Fig. 9. *Pteridium aquilinum.* Part of the spore mother cell showing nuclear vacuole (nv) and synaptonemal complexes (as at arrow). One inclusion similar in appearance to cytoplasm can be seen within the nucleus (*). Note also the rudimentary differentiation of the cytoplasmic organelles. Bar = 1 μm.

Other features unique to pteridophyte sporogenesis include the formation of nucleoloid-like bodies in the cytoplasm of the spore mother cells, and the dispersal of these bodies in the cytoplasm of the young spores concomitant with a replenishment of the ribosome population. Also noteworthy is a brief, but striking period of activity of the nucleus in young spores. In *Pteridium* this takes the form of narrow tubular extensions of the nucleus into the cytoplasm (Sheffield & Bell, 1979; see also Fig. 10) and in *Psilotum* and *Anemia* in the formation of invaginations of the nuclear envelope. In *Anemia* the invaginations are narrow and terminate within the karyoplasm in a bulbous swelling (e.g., Fig. 11, and see Schraudolf, 1984), often intimately associated with chromatin. These formations in *Anemia* and *Psilotum* correspond in timing and form with the nuclear invaginations characteristic of the young pollen grains of the gymnosperms *Pinus* (Dickinson & Bell, 1972) and *Podocarpus* (Aldrich & Vasil, 1970). In both these instances the invaginations are surrounded by chromatin, and the narrow portion contains osmiophilic material, shown in *Pinus* to be rich in RNA (Dickinson & Potter, 1975). The formation of the invaginations may mark the time at which the haplophase nucleus begins to impose gametophytic form and function upon the spore.

Wall formation within the tetrad is probably simultaneous in all homosporous pteridophytes, even in those instances where the spores have bilateral symmetry. The residence of the four spore nuclei for a brief period in a common cytoplasm may facilitate uniform early development of the spores. There would be an opportunity for any alleles with deleterious effects on spore formation to be compensated for during the coenocytic phase (Verma & Khullar, 1976).

In ferns showing apogamy of the kind found in *Dryopteris borreri* (*D. affinis*) the production of spores with the same chromosome number in the parent is brought about by the formation of a restitution nucleus before meiosis. The spore mother cell nevertheless closely resembles that of a sexually reproducing fern such as *Pteridium* (Sheffield et al., 1983).

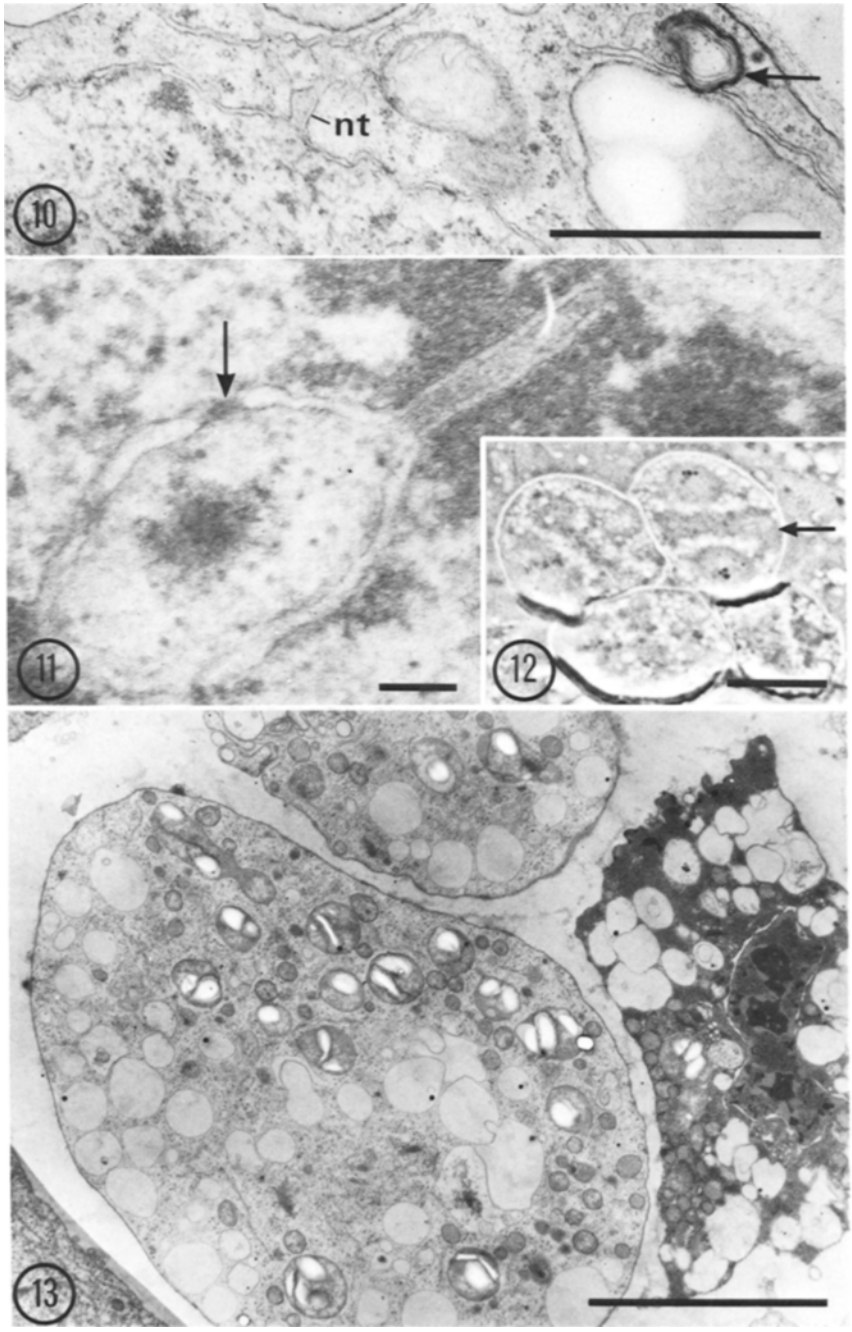
→

Fig. 10. *Pteridium aquilinum.* Young spore. Note protrusion of the nucleus (nt) bounded by both membranes of the envelope (nuclear tube). Bar = 1 μm.

Fig. 11. *Anemia phyllitidis.* Young spore showing invaginations of the nuclear envelope. The envelope of the invagination contains pores (as at arrow). Electron micrograph by H. Schraudolf. Bar = 0.1 μm.

Fig. 12. *Marsilea vestita.* Diads in the megasporangium. The organelles are confined to the equatorial plane (arrow) following the first division of meiosis. Resin section, phase-contrast. Bar = 20 μm.

Fig. 13. *Marsilea vestita.* Megaspores before release from the tetrad. One megaspore has already begun to degenerate (right). Bar = 5 μm.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In the 'diploid' strain of *D. borreri* examined, a unique feature was the centripetal development of a cell wall in the equatorial plane sometimes even reaching and indenting the nucleus. At this point the development stopped, and the wall was subsequently resorbed. Manton (1950), detecting such walls in light microscope preparations, thought them to be characteristic of 'imperfect' and unproductive sporangia. In the strain investigated by Sheffield et al. (1983), they appeared to occur regularly, and to interfere in no way with sporogenesis. This peculiar feature would repay investigation in other obligately apogamous ferns. The ingrowth evidently represents an abortive attempt at cross wall formation, but the manner in which it occurs is quite different from the formation of the wall in normal pteridophyte cell division, and recalls the situation in certain algae (Fowke & Pickett-Heaps, 1969).

B. HETEROSPOROUS SPOROGENESIS

Microsporogenesis in *Marsilea, Selaginella* and *Isoetes* appears to follow the same path as sporogenesis in homosporous pteridophytes, but few instances have been investigated at ultrastructural level. Observations on *Selaginella sulcata* (Pettitt, 1977) have produced evidence of an unequal distribution of ribosomes among the four spores of a tetrad. This may account for a variation in size of the microspores, and for the abortive spores found in many sporangia. Herd et al. (1985) revealed that in *Azolla microphylla* the wall of the microspore mother cell disappears, presumably as a consequence of enzymic digestion, as the nucleus enters prophase. At zygotene the mother cell is represented by a protoplast lying in a vacuole surrounded by plasmodial tapetum. Following meiosis the individual spores become surrounded by exine. Finally the plasmodial tapetum loses all cytoplasmic organization and its place is taken by a membranous lattice which forms the skeleton of the massulae.

In all investigations of microsporogenesis hitherto it has been impossible to identify features which can be regarded as significant in limiting the growth of the microspore to the production of a single, or pair of antheridia. This limited potentiality for development is no doubt related to the small amount of storage material in the microspore and the lack of photosynthesis, but the stage at which the ability to develop a photosynthetic system is lost is not known.

From the point of view of the causal factors in the life cycle, the events in megasporogenesis are probably more significant than those in microsporogenesis. That which induces femaleness in the megaspore can also be expected, by analogy with the life cycle of the homosporous pteridophytes, to prepare for the re-establishment of the sporophytic phase of growth. The simplest situation is seen in *Isoetes*. The megasporangia are

borne on the leaves formed at the beginning of a season's growth, and the microsporangia on those appearing later. The megasporangia contain fewer sporogenous cells than the microsporangia, and probably correspondingly more tapetum, although this awaits confirmation. All the meiotic products in the megasporangium develop into megaspores. Although the full course of megasporogenesis in *Isoetes* has yet to be investigated by modern techniques, Pettitt (1976) has discovered that the plastids in *I. engelmanni* go through a remarkable cycle at meiosis. The megaspore mother cell contains four disc-shaped proplastids up to 12 μm in diameter, one of which passes to each spore. These large proplastids have an irregular surface and appear to bud, yielding small bodies in the cytoplasm. This process continues in the young spores until, as a result of the continued fission, the cytoplasm of each is populated by normal proplastids and the original large proplastid has disappeared. In two other species of *Isoetes, I. pantii* and *I. coromandelina,* there are examples of sporangia in which both megaspores with normal trilete symmetry, and microspores with normal monolete symmetry are found together (Singh et al., 1983; Goswami, 1975a). There are as yet no detailed records of the viability of the spores from mixed sporangia. Since microsporangia are very rare in *I. coromandelina,* it is conceivable that the male gametes are derived principally from the microspores in the mixed sporangia. The situation is complicated by some populations of *I. coromandelina* being triploid and presumably apogamous, where only megasporangia are produced. In these the nucleus of the megasporocyte divides only once without reduction, yielding two triploid diad nuclei. The large plastid, however, divides twice and the cytoplasm cleaves such that four spores are produced, each containing a plastid, but only two of the spores contain a nucleus (Verma, 1960). In *I. pantii* a large acrocentric univalent appears always to be present at meiosis in the mixed sporangia (Goswami, 1975b).

In *Selaginella* megasporogenesis follows a different course. Usually only one megaspore mother cell is functional, but occasionally two or more may be present (Pettitt, 1971). It has been noted in *S. pilifera* that no callosed wall forms around the non-functional megaspore mother cells, and staining indicates that they contain little cytoplasmic RNA (Horner & Beltz, 1970). In *Selaginella sulcata* Pettitt (1977) has also distinguished two kinds of megaspore mother cells; the larger with a diameter of about 15 μm, and the smaller with about half this diameter and a cytoplasm poor in ribosomes and organelles. The smaller megaspore mother cells ultimately degenerate. Of the larger only one megaspore mother cell divides; the remainder gradually regress as the megaspores mature. The megaspores themselves differ in size, one pair being larger than the other. Germination tests have shown that the small spores are sterile (Pettitt, 1977). This difference in spore size comes about despite the equal par-

titioning of the spore mother cell, and it must therefore reflect a tendency for two spores of the tetrad to develop faster than, and possibly at the expense of, the other two. Pettitt was able to detect a gradient in the density of the cytoplasm in the mother cell, and proposed that the unequal behavior of the spores was a consequence of their being unequally endowed with cytoplasmic components. Although this is plausible, it is conceivable that, as in *Marsilea,* equality is established at diad formation, and the failure of two of the spores to grow follows from the segregation of nuclear factors at the first division of meiosis.

Amongst the ferns *Marsilea vestita* has been investigated in greatest detail (Bell, 1981, 1985). Eight spore mother cells become distinct at the center of the megasporangium. Almost immediately the envelopes of the plastids in the spore mother cells cease to react with osmium, and in many instances the stroma appears to be continuous with the ground cytoplasm. The mitochondria and plastids often lie to one side of the mother cell (probably indicating the position of the pole of the previous mitosis), but they move to the equatorial plane in the diad formed at the first division of meiosis (Fig. 12). At this time the envelopes of the plastids, which regain a normal reactivity with osmium, are associated with membranous tubules about 35 nm in diameter, apparently a specialized form of endoplasmic reticulum. These tubules persist until the spores are formed, but are no longer seen after the spores have separated and the exine has begun to appear. Only one spore survives in each tetrad; the degeneration of the remainder begins before the spores separate (Fig. 13). Of these eight surviving megaspores only one comes to maturity. This is always found in the tetrad which lies closest to the attachment of the sporangium. This megaspore grows rapidly, probably because of its proximity to inflowing nutrients. The development of the seven remaining megaspores is suppressed, and ultimately they become represented only by fragments of exine in the plasmodial tapetum. During meiosis the megasporangium increases its volume about twenty-four times, and a fluid-filled cavity appears at its center. The single developing megaspore grows into this cavity, which further enlarges by progressive digestion of the tapetum. Ultimately the megaspore occupies the whole of the sporangium, except for a thin layer of crushed tapetum at its surface which becomes mucilaginous on hydration. The growth of the megaspores is marked by conspicuous nucleocytoplasmic interaction similar to, but more extensive than, that seen in the maturation of the spores of the homosporous ferns. As in these, tubular extensions of the nucleus, sometimes branching, penetrate the cytoplasm. Although they have been followed for distances up to 10 μm, often running very close to mitochondria and plastids, no continuity has been detected between these tubes and any cytoplasmic organelles. This nuclear activity can be detected until the completion of

the wall of the spore prevents penetration of the fixative and satisfactory preservation of the cytoplasm. The wall of the mature spores of both *Marsilea* and *Azolla* (Herd et al., 1986) is complex, consisting of an unstructured exine component and a chambered outer layer (perine) formed at the periphery of the tapetum. The expansion of the growing spore brings the exine at its surface into contact with the perine, and the two then become firmly fused, presumably by the polymerization of sporopollenin precursors.

In all the heterosporous Pteridophyta (as opposed to the anisosporous [Bell, 1979b], where there is no degeneration of meiotic products) the megaspores give rise to a minute gametophyte bearing a single or a few archegonia. There is no report of the expression of male sexuality. In some heterosporous forms there is greening of the female gametophyte (e.g., *Marsilea*), but it is not known whether there is photosynthesis. In any event, the nutrition of the gametophyte, and the resources which support the early development of the sporophyte, are presumably derived principally or entirely from the substantial food reserve of the megaspore. We have found no reference to evidence of mycorrhiza in sporophytes or gametophytes of heterosporous ferns.

VI. Aberrant Life Cycles

A. APOGAMY (APOMIXIS OF LOVIS [1977])

Apogamy in the Pteridophyta is of two kinds, obligate and facultative. Obligate apogamy describes the kind of cycle in which a sporophyte is produced regularly from the gametophyte without sexual fusion. Although, as indicated earlier, sporogenesis is well understood in such plants, little is known about the factors which prevent the sexual function. The gametophytes of obligately apogamous species often produce perfect antheridia, and the spermatozoids are capable of fertilizing the eggs of related sexual species. Archegonia, if they are produced, do not function, and this has been attributed to the 'poor development' or 'necrosis' of the egg cells (see, for example, Gastony & Haufler, 1976; Nayar & Bajpai, 1968). Archegonia of *Pteris cretica,* however, contain egg cells which appear well developed (e.g., Fig. 1), and which may be unable to function solely as a result of the necrosis of the topmost archegonial neck cells (Laird & Sheffield, 1986). The experimental and ultrastructural examination of oogenesis in *P. cretica* currently in progress should provide firm evidence about the integrity and viability of the egg cells.

In obligately apogamous ferns the sporophytes arise most frequently from somatic cells located behind the apical meristem (the site in many ferns of the initiation of archegonia), but sometimes (as in Fig. 14) elsewhere in the gametophyte. Approximately 10% of ferns, and an unknown

proportion of other pteridophytes, have cycles of this kind. The initiation of sporophytic outgrowths does not involve necrosis of adjacent prothallial cells, or the laying down of a boundary separating the sporophytic growth from the gametophyte (Laird, 1986).

Facultative apogamy can be induced in a number of ways, usually involving manipulation of the environment. Depriving gametophytes of water (thus preventing the opening of archegonia, and release of spermatozoids) is often effective (e.g., *Lycopodium,* Freeberg, 1957; and many ferns, Farlow, 1874). High levels of illumination also promote apogamy (e.g., Lang, 1898), possibly by increasing the general level of photosynthesis in the cells. Whittier and Steeves (1960) found that high concentrations of glucose, sucrose, fructose and maltose led to an increased frequency of apogamous outgrowths in ferns, and attributed this partly to a metabolic and partly to an osmotic effect. Sugars were not found to promote apogamy in *Equisetum,* however, although kinetin was effective (Ooya, 1974). Ethylene has also been shown to promote apogamy in ferns. Elmore and Whittier (1975) recognized two stages in the induction of facultative apogamy in *Pteridium.* The first stage required both high carbohydrate levels and ethylene, the second only carbohydrate. The increased frequency of apogamy in crowded fern cultures can be attributed to an accumulation of naturally produced ethylene. The manner in which promotion of apogamy is brought about by ethylene, kinetin or other metabolites is still wholly unknown.

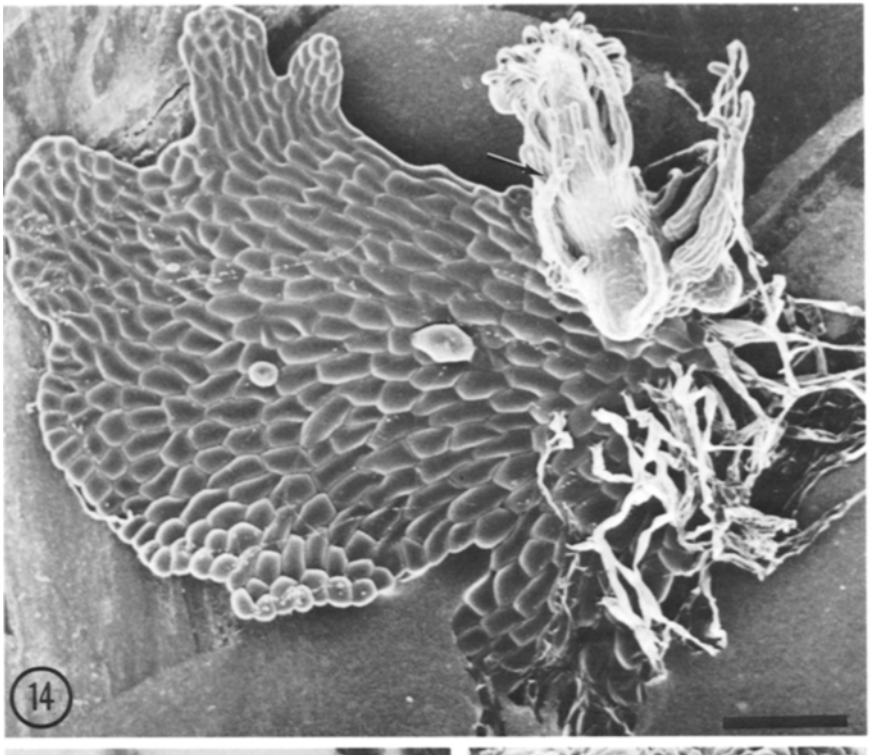
The absence of fertilization during apogamous reproduction results in the reproduction of the same genome, generation after generation and in the nuclei of gametophyte and sporophyte having the same chromosome number. In instances of facultative apogamy where the sporophyte is capable of prolonged growth (as in *Scolopendrium* [Manton, 1950] and *Dryopteris* [Manton & Walker, 1954]) no restitution nucleus is formed and meiosis is abortive. The production of a very few viable spores in such instances as a consequence of chance events in meiosis cannot be ruled out. If such spores were found, breeding from them might give valuable information about the genomic constitution of the parent species. In obligate apogamy sporogenesis occurs regularly, but sporophyte and gametophyte retain the same chromosome number. This is usually achieved either by the formation of a restitution nucleus before meiosis, so that the nuclei of the spores contain the original number of chromosomes, or by asynapsis and the formation of a restitution nucleus in the first division of meiosis. This leads to a diad in which the nuclei contain an unreduced chromosome number. In most obligately apogamous ferns the restitution nucleus forms immediately before meiosis, but Hickok (1979) described a remarkable mutant stock of *Ceratopteris* in which it occurs much earlier during vegetative growth.

B. APOSPORY

In contrast to apogamy, aposporydoes not occur regularly as part of a life cycle. The only recorded instance of apospory in the field (Farlow, 1899) is now known to be associated with mite infestation (Whittier, 1966). Nevertheless, aposporous gametophytes may occur occasionally in Nature. Since such gametophytes are commonly normal and fully functional sexually, they may account in part for the polyploidy common in ferns, although the production of spores with unreduced chromosome numbers (e.g., Gastony, 1986) is probably the principal cause. A remarkable instance of spontaneous apospory was the 'peculiar' *Phyllitis scolopendrium* of Anderson-Kötto (1932) in which the leaves produced gametophytic outgrowths without any experimental treatment. This behavior, which was associated with stunted morphology, appeared to be governed by Mendelian genes. The plant has unfortunately been lost, and the genetics remain unresolved.

Detailed studies of pteridophyte apospory under laboratory conditions have been confined to the ferns. The induction of apospory usually requires the removal of organs or parts of organs from the remainder of the plant, but gametophytes have occasionally been observed on attached leaves or roots, provided these are in contact with a moist surface (Hurel-Py, 1950; Munroe & Sussex, 1969; Sheffield & Attree, 1983; Sheffield & Bell, 1981a). In *Pteridium* it has been shown that regions of leaves which behave in this fashion have lost vascular continuity with the remainder of the plant (Sheffield & Bell, 1981b). This interruption of the vascular supply, which appears to involve occlusion of the tracheids by deposits of an unknown osmiophilic material (Sheffield et al., 1982), may have causal significance. It is possible that substances suppressing the meristematic activity of the cells of the lamina and/or stabilizing the sporophytic condition enter the leaf through the xylem. Cutting off the supply of these substances by excision or occlusion of the tracheids may allow the resumption of meristematic activity. The situation in which gametophytes arise from roots (Fig. 15) has not yet been fully investigated.

The other necessary condition for apospory, contact with a moist surface, may provide the opportunity for those regulatory substances still present in the tissue to diffuse into the substrate. This concept is strengthened by the finding that the more hydrated the medium supporting the explants, the greater the response (Sheffield & Bell, 1981a; von Aderkas, 1986), the exact converse of the relation between hydration and apogamy. The reluctance of older leaves to yield aposporous outgrowths has been attributed to the thicker cuticle restricting the outflow of regulatory substances (Sheffield & Bell, 1981a). It has now been shown that leaves produced in liquid medium are increasingly incapable of apospory; those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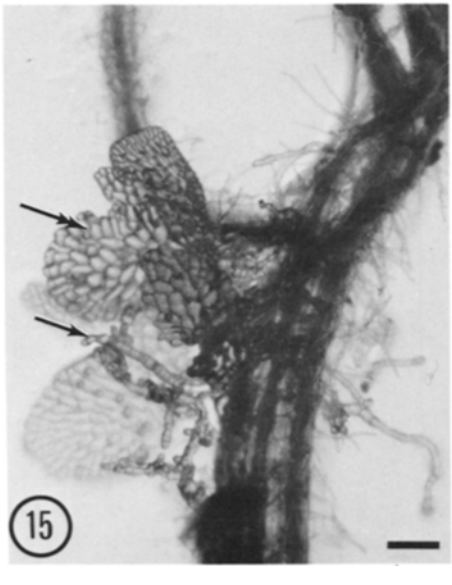
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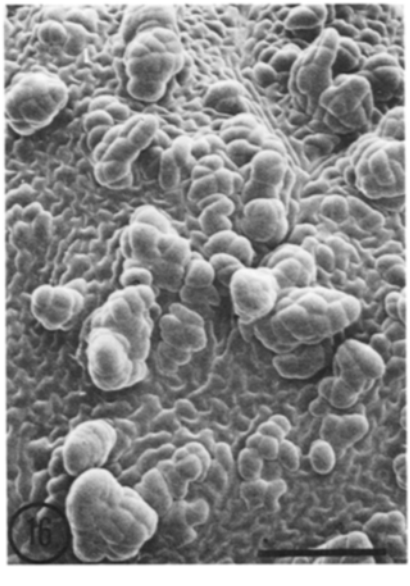
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after the seventh formed yield no outgrowths (von Aderkas, 1986) as found with *Pteridium* raised on solid medium (Sheffield & Bell, 1981a). As it is unlikely that leaves produced in liquid culture bear significant amounts of cuticle it may be that the sporophytic condition becomes progressively more firmly established as development proceeds, as envisaged by Takahashi (1962).

In the experimental system of Sheffield and Bell (1981a) the first leaves of sporelings were excised and the laminae bisected. The portions of lamina were then placed on a mineral-agar medium. In these conditions numerous aposporous outgrowths from the surface and margins of the explant could be detected within three days of excision. The presence of sucrose in the medium effectively suppressed successful apospory. This was clearly not an osmotic effect since equivalent concentrations of mannitol had no influence on the response. Sucrose apparently tends to maintain the sporophytic nature of the tissue, and to discourage gametophytic growth, in line with the earlier results of Hirsch (1975). The experiments of Sheffield and Bell also indicated that conditions which induced apospory in juvenile leaves of *Pteridium* and *Thelypteris* were wholly ineffective in causing proliferation of any kind in comparable leaves of *Marsilea vestita*. Although it may prove possible to induce apospory in homosporous pteridophytes other than ferns (the experiments have yet to be made) the present indications are that, with the transition to heterospory, the capacity for aposporous development was lost. The evolution of heterospory was evidently accompanied by a stabilization of the sporophytic condition.

It has been generally held that aposporous outgrowths arise from cells isolated from general correlative influence by death of adjacent cells (Bell, 1979b). In the instance of outgrowths from starved roots this appears to be regularly so (Munroe & Bell, 1970). In the experimental system of Sheffield and Bell, however, there is no evidence from fluorescence microscopy, scanning electron microscopy (Fig. 16; see also Sheffield, 1984a, 1985), or transmission electron microscopy of any isolation of the cells

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Fig. 14. *Pteris cretica.* Cryo-scanning electron micrograph of gametophyte bearing an apogamous sporophyte (arrow). Note the lack of a distinct meristematic region in the gametophyte. Bar = 400 μm.

Fig. 15. *Pteridium aquilinum.* Photomicrograph of a root attached to the parent plant in culture. Note the profusion of aposporous outgrowths, both filamentous (arrow) and cordate (double arrow). Bar = 500 μm.

Fig. 16. *Pteridium aquilinum.* Cryo-scanning electron micrograph of the lamina of a juvenile leaf detached from the parent and cultured for five days. Numerous aposporous outgrowths can be seen arising from the upper surface. Bar = 300 μm.

which produce aposporous outgrowths, or of any cell death. Here it appears that all the cells of the explant (with the significant exception of the guard cells) are capable of generating outgrowths. Closer consideration shows that this discrepancy is apparent rather than real. In the experiments of Munroe and Sussex (1969) collars of cells sloughed off from the tips of roots cultured in starvation conditions also yielded gametophytes. These collars are equivalent to multicellular explants. Isolation from correlative influences can therefore be achieved either by the death of cells around a surviving cell, or by spontaneous or experimental excision of tissue. In conditions of stress generated by metabolic deficiency and leaching, isolated sporophytic cells or groups of cells will, given conditions adequate to support autotrophic growth, grow out as gametophytes. The few instances of apospory on attached leaves can also be accommodated in this concept if, as seems likely, the effects of metabolic deficiency and leaching are additive. The blockage of the tracheids observed in *Pteridium,* if of general occurrence, would provide a measure of metabolic deprivation. Vigorous leaching can be envisaged as overcoming the effects of the cell contacts which still remain.

Guard cells are a conspicuous exception to the general principle of isolation. Vital staining shows that these cells remain fully alive in leaf explants, despite their lack of aposporous activity. This could be attributed to their differentiated state, or the explanation may lie in the thickened walls of guard cells. These walls, often with several distinct layers, may be callosed or even lignified (Waterkeyn & Bienfait, 1979), and leaching, in consequence, effectively impeded. This might account for the sporophytic state of the cell being retained despite isolation of the laminae. Further studies of the cytochemistry and ultrastructure of guard cells are clearly called for since the sharp difference in their behavior from that of epidermal cells generally in relation to apospory raises problems of fundamental importance.

VII. Causal Factors in the Life Cycle

The ultrastructural and cytochemical data relating to gametogenesis and sporogenesis, and the experimental data relating to apogamy and apospory, now make it possible to identify the factors likely to control the phasic alternation in the life cycle of the pteridophytes.

With regard to the transition from gametophyte to sporophyte it is reasonable to examine gametogenesis for indications that this is the time at which the sporophytic genes are activated, or when the cellular environment is generated in which activation can occur. Spermatogenesis seems very unlikely to be significant in this respect. The extreme condensation of the chromatin suggests that the male genome at this time is

inactive. The cytoplasm which remains after differentiation seems wholly concerned with providing motility, and subsequently a supply of tubulin to the egg cell and ultimately the zygote (Bell, 1986). The cytoplasm of the egg cell, by contrast, is a rich storehouse of DNA (Sigee & Bell, 1971) and RNA (Cave & Bell, 1974a) and interference with the RNA disturbs subsequent sporophyte development (Jayasekera & Bell, 1972). The evidence therefore points to the new phase of sporophyte growth having already been initiated in the egg cell. Fertilization restores the ability of the cell to divide, and the expression of the sporophytic genes becomes possible.

When extensive nucleo-cytoplasmic interaction was first discovered in eggs of *Pteridium* and other ferns, it was natural to propose that this marked the time at which a re-programming of the cytoplasm for sporophytic growth occurred (Bell, 1979b). There may be a coincidence, but active penetration of the cytoplasm by nuclear protrusions is not essential for a normal life cycle. As stated earlier, this feature is absent from the egg cells of many pteridophytes. Also, in *Pteridium,* the production of nuclear protrusions continues no less actively in the presence of thiouracil, despite the destabilization of the sporophytic nature of the subsequent embryo (Bell, 1972). In those species in which nucleo-cytoplasmic interaction occurs, the cytology is surprisingly similar to that seen in the cells of tobacco infected with virus (Wettstein and von Zech, 1962). This raises the interesting possibility that many leptosporangiate ferns carry symptomless viruses, the presence of which is revealed only during oogenesis.

The presence, described earlier, of numerous nuclear bodies, and of bodies of similar size, texture and composition in the cytoplasm, is another equivocal feature of the egg cells of several pteridophytes. The acidic protein of which the bodies consist may be a structural protein of the chromosomes temporarily displaced during the fine dispersal of the chromatin in the egg nucleus. These bodies may play a role in the rapid decondensation of the male chromatin when it comes into contact with the female cytoplasm (Bell, 1975) and in the reconstitution of the chromosomes of the zygotic nucleus as it prepares for prophase. There is no evidence that they play any part in bringing about the change of phase.

In the search for determining factors in oogenesis we must therefore look to more general features of the archegonial sequence. Oogenesis always involves substantial plasmatic growth, most of the cytoplasm, despite the presence of several nuclei in the canal, remaining with the egg cell. The final maturation of the egg cell takes place in an archegonial chamber surrounded by a thickened wall lacking plasmodesmata, and is accompanied by the dissolution of the neck canal cells and the shrinkage of the ventral canal cell. Lysis is now likely to lead in the confined space of the sealed archegonium to a high concentration of nutrients and par-

tially depolymerized macromolecules. The egg cell, which is only just beginning to acquire its acetolysis-resistant extra membrane (Cave & Bell, 1974b), thus has an opportunity, enjoyed by no other cell in the life cycle of a homosporous pteridophyte, to take in these extra metabolites in abnormal amounts. It seems likely that the peculiar cytochemical features of the egg cells of ferns, noted earlier, are a consequence of this inflow of metabolites, and will be found to be characteristic of the egg cells of pteridophytes generally as maturation is completed. Further, it is proposed that this enrichment of the cytoplasm of the female gamete initiates the activation of the genes responsible for sporophytic growth, and this in turn leads to messenger RNA's appropriate to sporophytic growth being already present in the cytoplasm at the time of fertilization.

If the final stage of maturation of the egg cell of a homosporous pteridophyte has the features proposed, the inflow of lytic products appears also to be potentially disruptive. In *Pteridium,* for example, the viability of mature egg cells is limited, probably extending for no more than a few hours (Bell & Duckett, 1976). This may account for the extreme rarity or absence of parthenogenesis in homosporous pteridophytes (there is no convincing evidence of any sporophytic outgrowths originating from egg cells). The reluctance of the unfertilized egg cell of homosporous ferns to divide may be related to the highly dispersed state of the chromatin. The re-establishment of the integrity of the chromosomes may not be possible until the entry of the condensed chromatin of the male gamete. A contributory cause in ferns such as *Pteridium* and *Dryopteris* is possibly the absence of microtubules. It is conceivable that in these instances the tubulin genes are inactive in the egg cell and that no tubulin is available for the formation of a spindle. The entry of the spermatozoid with its numerous axonemes would naturally correct this deficiency. This hypothesis, however, loses force in view of the occurrence of microtubules in the egg cells of *Anemia* and *Todea,* in neither of which is parthenogenesis reported.

Considerations of cytoplasmic enrichment also apply to the anisosporous and heterosporous pteridophytes. The establishment of femaleness can be taken as the first step towards the conditions which lead to the activation of sporophytic genes. Femaleness, of spores as well as egg cells, appears to be invariably associated with elaboration of the cytoplasm. Studies of the expression of sexuality in the anisosporous pteridophytes (e.g., *Platyzoma* [Tryon, 1964]; *Ceratopteris* [Schedlbauer, 1976] and *Equisetum* [Duckett & Pang, 1984]) indicate that the more abundant cytoplasm of the larger spores is correlated with the direct expression of femaleness. In *Isoetes,* as in *Platyzoma,* large spores result from the intervention of few mitoses in the archaesporium before the onset of meiosis. Although the female gametophyte of *Isoetes* is heterotrophic, it is not

impossible that it could be cultured in vitro. Further growth of the gametophyte might then lead, as with the initially female gametophytes developing from the large spores of *Platyzoma,* to a male phase. In *Selaginella* and *Marsilea,* in addition to there being fewer megasporocytes than microsporocytes, and correspondingly more tapetum associated with the former, there is enrichment of the milieu in which a viable megaspore develops by the resorption of either some of the megasporocytes or of meiotic products. In *Marsilea vestita* the one functional megaspore is particularly well provided with the nutritional reserves in the form of carbohydrate and lipid. Here oogenesis occurs in such nutritionally rich conditions that, in the absence of fertilization, the egg cell readily gives rise to a sporophyte parthenogenetically (Mahlberg & Baldwin, 1975). In heterosporous plants the activation of the sporophytic genes probably begins in the maturation of the megaspore.

The experimental induction of apogamous outgrowths has so far contributed little to an understanding of the factors effective at a cellular level in bringing about the change of growth. The many experiments of Whittier and his students have shown that the effect is promoted by enhanced nutrition (see Elmore & Whittier, 1975). However, since neither the timing nor the position of apogamous outgrowths can be predicted with certainty, the search for significant ultrastructural and cytochemical changes which might be causally related to the change in growth cannot be concentrated on any particular cell of the gametophyte. Even in obligately apogamous ferns, such as *Pteris cretica,* the origins of sporophytic outgrowths are unpredictable (Laird, 1986), so they are similarly unhelpful as far as such studies are concerned. It is possible that the use of techniques, as developed, for example, by Attree and Sheffield (1985), for the isolation and culture of gametophytic protoplasts might provide a more clearly defined system for the study of apogamy.

The lack of discontinuity between apogamous outgrowths and the surrounding gametophytic tissue is a situation quite different from that in sexually reproducing ferns (in which there is always a clear boundary between the embryo and the gametophyte). It is possible that the cells of the gametophyte of an obligately apogamous fern are already partially sporophytic, and that the full expression of the sporophytic genes becomes possible at locally favored sites. If, in such ferns, the sporophytic genes become partially activated in sporogenesis, this too would be in line with the hypothesis of cytoplasmic enrichment (Bell, 1983c). Apogamous meiocytes, as a consequence of the formation of the restitution nucleus and the cell failing to cleave, contain considerably more cytoplasm than the corresponding cells of a sexual relative, and the spores are consequently larger. The gametophytes of apogamous ferns mature faster than those of the sexual (Whittier, 1970). The apogamous ferns, from the point of view

of causal factors in the life cycle, may therefore occupy a place intermediate between the homosporous and the heterosporous. The augmented cytoplasm of the spore can be envisaged as partially activating the sporophytic genes. Activation is completed during the brief gametophytic phase in a manner not yet understood, and the transition to firmly sporophytic growth follows. It will be particularly interesting to see whether, in consequence of the potentially sporophytic nature of the gametophyte, the oogenesis which occurs regularly in certain races of the apogamous *Pteris cretica* (Fig. 1; see also Laird, 1986) differs significantly in ultrastructural and cytochemical features from that in its sexual relatives.

In considering the change from sporophyte to gametophyte, attention naturally turns to sporogenesis. The loss of RNA from the cytoplasm in the prophase of meiosis closely resembles the situation in *Lilium* (Dickinson & Heslop Harrison, 1977), who regard this feature as having causal significance in relation to the change of phase. The discovery that microsporogenesis in certain seed plants is not accompanied by appreciable loss of cytoplasmic RNA (Pennell & Bell, 1986, 1987) challenges this hypothesis. The extent of the depolymerization of the cytoplasmic RNA may be related instead to the speed with which meiosis is accomplished.

The ability to pass directly from sporophyte to gametophyte by way of apospory provides the opportunity to study the change in form without the complication of meiosis. The investigation of the cells giving rise to aposporous outgrowths in the experimental system of Sheffield and Bell (1981a) has revealed the striking fact that the cytoplasm in no way resembles that of spore mother cells. There is no dedifferentiation of mitochondria or plastids, or reduction in the number of ribosomes, aspects of the cytoplasm of meiotic cells which have been considered by Dickinson and Heslop Harrison (1977) to be significant in relation to the change of phase. On the contrary, as the cells regain meristematic activity the cytoplasm becomes denser, the vacuoles reduced in volume, and the number of organelle profiles increases. A phase change from sporophyte to gametophyte can thus occur, even in species in which this is a normal feature of meiotic prophase, without the drastic purging of the cytoplasm envisaged by Dickinson and Heslop Harrison.

Although consideration of the conditions which promote apospory points to metabolic impoverishment, by either deprivation or leaching, or both, as a cause of the switch in gene activation from sporophytic to gametophytic, separation from the correlative influences of the intact plant is also necessary. In the experiments of Munroe and Sussex (1969) it was clearly demonstrated that an increase in the concentration of simple nutrients was not adequate to maintain the activation of the sporophytic genes. Supplementation of the medium with ammonium ions, casein hydrolysate and kinetin promoted the aposporous response from roots,

presumably by stimulating meristematic activity. The influence from the parent plant which normally suppresses the aposporous response is therefore likely to be macromolecular in nature, and not a simple nutrient.

The hypothesis that metabolic impoverishment brings about the transition from sporophyte to gametophyte is applicable also to sporogenesis. Although the evidence is not yet conclusive, it seems likely that, as in *Lilium* (Heslop Harrison & Mackenzie, 1967), the presence of the thickened wall around the spore mother cell is correlated with a diminished uptake of complex molecules. With respect to the availability of complex molecules the situation of the mother cell corresponds to that of an explanted somatic cell. The correlative influence of the parent plant is removed, and the cell suffers metabolic deprivation. This can be regarded as the primary cause of the switch in gene activation. The ensuing cytochemical changes in the cytoplasm, which involve depolymerization of RNA, either massive, as in *Lilium* (Mackenzie et al., 1967), or restrained, as in *Taxus* (Pennell & Bell, 1987), are probably concerned with the demands of the nucleus in meiotic prophase, but they will complement the metabolic stress to which the cell is subjected. Subsequently the cytoplasm, which has not been augmented by plasmatic growth, is divided amongst the four spores. The spore is therefore a cell produced in deprived conditions and with a relatively depleted cytoplasm. It is the exact converse of an egg cell, although both egg cell and spore contain a haploid nucleus.

Only in the heterosporous Pteridophyta is this sequence modified. Although heterosporous microsporogenesis closely resembles homosporous sporogenesis, the fewer and larger megaspore mother cells accumulate more cytoplasm before being isolated by their thickened walls. The consequences of the impoverishment the cell suffers during meiosis are effectively reversed by the resorption by the surviving spores of the materials released by the lysis of those which perish. It seems reasonable to regard the mature megaspore as already partially sporophytic as a consequence of the stimulating effect of this inflow on the genes determining reproductive phase.

VIII. Conclusion

It will be evident that the Pteridophyta have much to offer those studying causal factors in the life cycle of the land plants. It is clear that developments in ultrastructural immunohistochemistry will soon facilitate the use of probes to detect the times at which the proteins characteristic of the ensuing phase begin to appear when one phase is changing to the other. Likewise the use of specific DNA probes will make it feasible to detect the instances of gene reactivation and suppression initiating the

phase change. There is thus a real prospect of being able to explain the alternation of phase in the land plants in molecular terms, and seeing it as the inevitable consequence of progressive changes in the physics and chemistry of cells. Clearly the Pteridophyta stand out amongst the vascular plants as the most suited for penetrating investigation by the powerful new techniques which are now becoming available.

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